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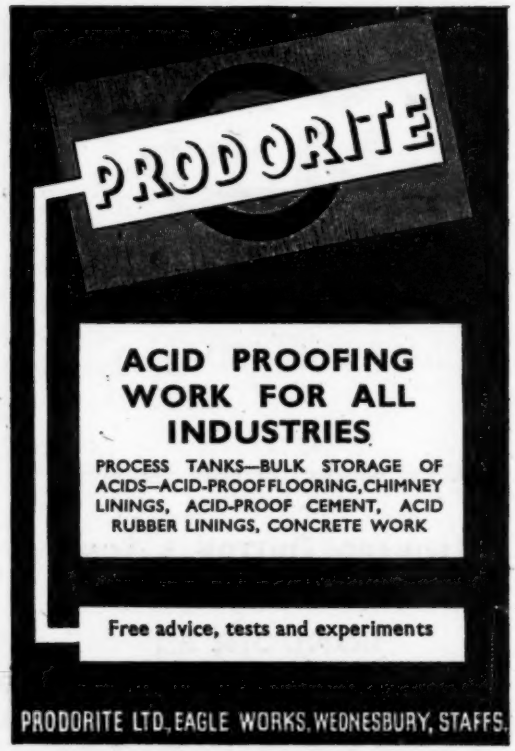
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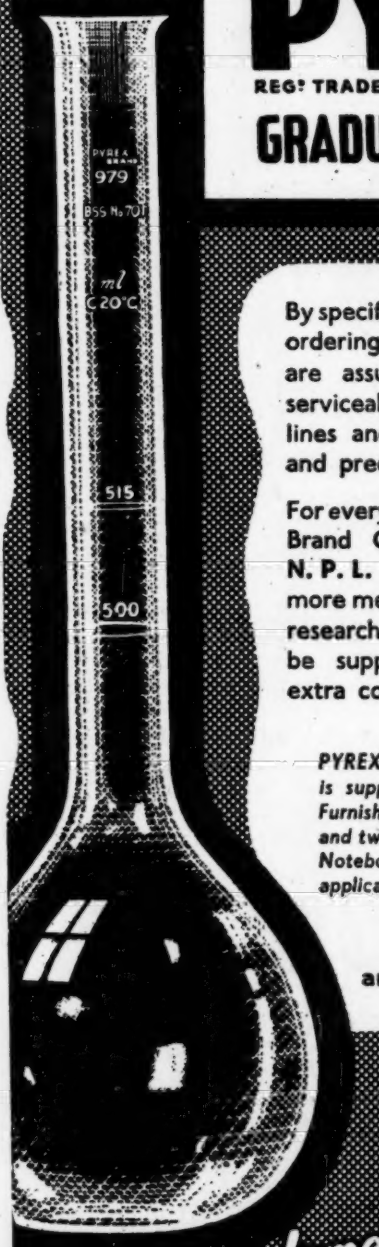

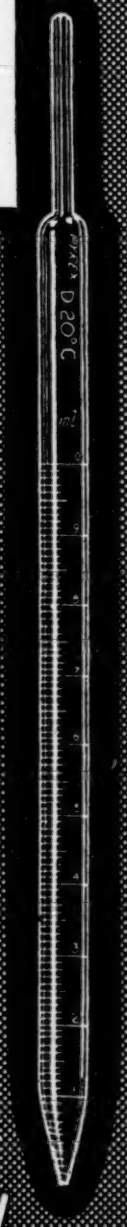

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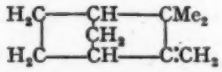
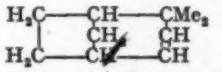
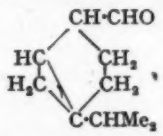
gave on treatment with chromyl chloride in carbon disulphide solution a substance $C_{10}H_{16}.2CrO_2Cl_2$, decomposed by water to yield an aldehyde, $C_{10}H_{16}O$, and a ketone, $C_9H_{14}O$, together with a chloro-compound, $C_{10}H_{15}OCl$. The structure of these degradation products was not elucidated, but it is highly improbable that they contain either a pinane or a camphane ring structure. Many years later, in collaboration with Chisholm (J., **1924**, **125**, 106), he found that the ketone was formed also by the oxidation of β-pinene with chromyl chloride, although the other products were different and the only one identified was *trans*pinolglycol (Wagner and Slawinski, *Ber.*, 1899, **32**, 2072). The oxidation of bornylene with chromyl chloride (with Heilbron, J., 1911, **99**, 1887) proceeded more smoothly, yielding a chloro-ketone, $C_{10}H_{15}OCl$, from which camphoric acid was obtained on oxidation. This chloro-ketone, since it is not identical with either of the isomeric α-chlorocamphors, subsequently described by Lowry and Steele (J., 1915, **107**, 1382), is probably a chloro*epi*camphor. Other products of the oxidation were camphenilanaldehyde and *iso*camphanilanaldehyde. Henderson and Heilbron during the course of this research studied also the action of nitrous and nitric acids on bornylene. By the action of the former reagent bornylene nitrosite was prepared, and by the action of nitric acid dinitrocamphane was obtained. As a result of this investigation Henderson and Heilbron (J., 1911, **99**, 1901) were led to discuss in an interesting theoretical paper the structure of camphene, for which at that time the formulæ (I) and (II) were under consideration.

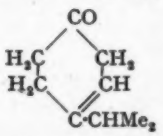
(I.) 

 (II.)

In this admirable contribution they advanced cogent evidence in support of (I) and they suggested, apparently for the first time, that the abnormal results obtained on the oxidation of camphene with potassium permanganate may have been due to the alkalinity of the solution in which the oxidation was carried out. This suggestion was subsequently confirmed by Langlois (*Ann. Chim.*, 1919, **12**, 301).

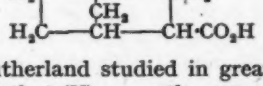
With Robertson and Brown (J., 1922, **121**, 2717; 1924, **125**, 265) the oxidation of sabinene with chromyl chloride was studied and was found to give sabinenaldehyde (III) and Δ^1-*iso*propyl*cyclo*hexen-4-one (IV).

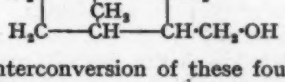
(III.) 

 (IV.)

The first action of chromyl chloride on monocyclic terpenes would appear to be their dehydrogenation to *p*-cymene, since it was found (J., 1907, **91**, 1871; with Cameron, J., 1909, **95**, 969) that both limonene and α-terpinene gave α-*p*-tolylpropaldehyde and *p*-tolyl methyl ketone, products identical with those obtained by the reaction of this reagent and *p*-cymene (Errara, *Gazzetta*, 1889, **19**, 528; 1891, **21**, 76; Miller and Rohde, *Ber.*, 1890, **23**, 1070; 1891, **24**, 1356).

In another series of papers the oxidation of the dicyclic terpenes α- and β-pinenes, camphene, and bornylene with hydrogen peroxide was described (with Sutherland, J., 1910, **97**, 1616; 1912, **101**, 2288; 1914, **105**, 1710; with Caw, J., 1913, **103**, 1543; with Chisholm, J., 1924, **125**, 107). With α- and β-pinenes, only the usual hydration products, α-terpineol, borneol, and fenchyl alcohol, were obtained, but results of greater interest were observed with camphene and bornylene. Oxidation of camphene resulted in the formation of camphene glycol, camphenilone, *iso*camphenilanaldehyde, an isomeride of camphenilanaldehyde (V), and camphenanic acid. Peculiar interest attaches to this acid, m. p. 95°, since it can be isomerised to *iso*camphenanic acid, m. p. 74°. These two acids are isomeric also with the camphenilanic acid, m. p. 65°, and *iso*camphenilanic acid, m. p. 118°, prepared originally by Bredt and Jagelki (*loc. cit.*) by the oxidation of camphene with chromyl chloride. Bredt (*Annalen*, 1909, **366**, 57) assigned to these acids the structure (V).

(V.) 

 (VI.)

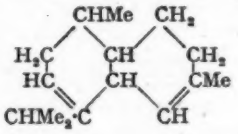
Henderson and Miss Sutherland studied in great detail the interconversion of these four acids, and, whilst there can be little doubt that (V) correctly represents camphenilanic and *iso*camphenilanic acids, the relationship of these four acids to one another is still obscure, since their esters give on reduction the same alcohol, camphenilanol (VI). Camphenanic and *iso*camphenanic acids are formed also by the oxidation of bornylene with hydrogen peroxide. A convenient method for the preparation of this hydrocarbon in the pure state was described by Henderson and Caw (J., 1912, **101**, 1416).

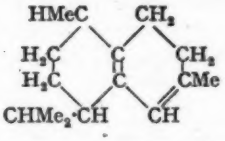
A brief reference may be made also to three papers (with Heilbron and Howie, J., 1914, **105**, 1367; with Marsh, J., 1921, **119**, 1492; with Kerr, J., 1924, **125**, 102) on the action of hypochlorous acid on α- and β-pinene and on camphene. With the former hydrocarbons ring fission occurs with the formation of dichlorohydroxymethanes; from camphene a chloro*iso*borneol is obtained.

Influenced doubtless by his interest in catalysis, Henderson synthesised also some *p*- and *m*-menthadienes (with Sutherland, J., 1910, **97**, 1616; with Boyd, J., 1911, **99**, 2159; with Schotz, J., 1912, **101**, 2563; with Smeaton, J., 1920, **117**, 144). Since the structures of these hydrocarbons were not determined, it will suffice to illustrate this aspect of Henderson's work by one example. Thymol was catalytically hydrogenated by the

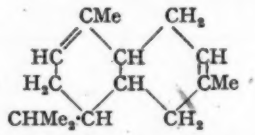
Sabatier–Senderens process to menthol, from which by dehydrogenation Δ^3-menthene was prepared. This on treatment with bromine gave a dibromide, from which by the action of alkali $\Delta^{3:4}$-*p*-menthadiene was obtained.

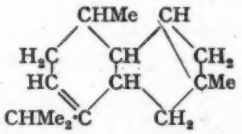
About 1924, Henderson entered the field of sesquiterpene chemistry. Here his attention was directed mainly to the hydrocarbons cadinene and the caryophyllenes. He found with A. Robertson (J., 1924, **125**, 1992) and with J. M. Robertson (J., 1926, 2811) that cadinene could be isomerised readily to *iso*cadinene, for which on the basis of the then accepted α-cadinene formula (VII) the structural formula (VIII) was suggested.

(VII.) 

 (VIII.)

The formula for cadinene (IX) now advanced (Campbell and Soffer, *J. Amer. Chem. Soc.*, 1942, **64**, 417) makes this isomerisation equally readily explicable.

(IX.) 

 (X.)

As Henderson and Robertson point out, *iso*cadinene is identical probably with a hydrocarbon isolated by Troger and Feldmann (*Arch. Pharm.*, 1898, **236**, 692) and by Lepeschkin (*J. Russ. Phys. Chem. Soc.*, 1908, **40**, 26) from oil of cade. Incidental to this investigation was the separation from the supa-oil (from *Sundora Wallichii*) of the hydrocarbon copaene (with McNab and Robertson, J., 1926, 3077). Since copaene is stated to give cadinene dihydrochloride on treatment with hydrogen chloride, it can probably no longer be represented by (X) and this new source of the hydrocarbon may render possible a reinvestigation of its structure. Henderson's final publications (with Robertson and Kerr, J., 1926, 62; with McCrone and Robertson, J., 1929, 1368; with Bell, J., 1930, 1971) were concerned with the chemistry of the caryophyllenes. Perhaps the most important contributions which he made were the observations that whereas α-caryophyllenic alcohol on dehydration gave solely clovene and on oxidation the crystalline acid, clovenic acid, the β-alcohol gave a mixture of clovene and *iso*clovene on dehydration and a liquid acid on oxidation.

ARTHUR AVERY READ.

1868—1943.

Prof. A. A. Read died on September 24th, 1943, at Bournemouth, where he had lived after his retirement from the Chair of Metallurgy at the University College of South Wales and Monmouthshire, Cardiff. He was born at Honiton, Devon, in 1868 and was educated at Exeter Grammar School, Owens College, Manchester, and at Sheffield University—then the Technical College—where he studied metallurgy under the late Prof. J. O. Arnold, F.R.S. He qualified as A.I.C. (1889) and F.I.C. (1895) and was subsequently awarded the degrees of M.Met. and D.Met. by the University of Sheffield. He went to Cardiff in 1894 as the first Lecturer in Metallurgy, and later, when metallurgy had been constituted as a separate department, was appointed the first Professor.

Read was the pioneer of metallurgical education in Wales. His enthusiasm was such that the new department flourished. He obtained University recognition for an Applied Science degree with metallurgy as the principal subject and thus, in 1903, had the distinction of having trained the first student in Great Britain to obtain the degree of B.Sc. in metallurgy. Before his retirement in 1933 he had fulfilled his ambition to establish a department worthy of the University of Wales, housed in a modern and well-equipped building which had been erected with the financial assistance of the Monmouthshire and South Wales Coal Owners' Association.

Read realised that the University had a two-fold obligation, not only to teach but also to prosecute original research, and he managed to keep an even balance between the two while at the same time he worked hard to build and equip his department. His work in collaboration with Arnold on the chemical and mechanical relations of iron, carbon and certain alloy elements in steel was published over a period of years from 1894 to 1915 and was an important contribution to the development of modern alloy steels. Following the lead of Abel and others (who used solution methods), Arnold and Read in 1894 devised the electrolytic method of separating Fe_3C from steel and later adapted it to the investigation of carbides occurring in alloy steels. It was with this aspect of the joint work that Read was primarily concerned. It is now generally agreed that analytical data regarding the composition of residues obtained by chemical attack should be used with caution in drawing conclusions as to the constitution of the carbides present in a steel. Nevertheless there were several instances in which carbides isolated by Arnold and Read showed the characteristics of a definite compound, *e.g.*, Cr_4C, V_4C_3, WC. The presence of these in steel has since been confirmed by *X*-ray analysis.

as also has the isomorphous replacement of iron in Fe_3C by manganese, cobalt, or nickel revealed by Arnold and Read's work, which thus remains a sound check on the constitution assigned to slowly cooled steels containing about 0·6 to 0·9% of carbon in the ternary equilibrium diagrams of carbon, iron, and another metal.

Read's interest in non-ferrous metallurgy resulted in the publication of several papers in the *Journal* of the Institute of Metals, 1915—1922, dealing with alloys of nickel, aluminium, and copper. The work on alloys containing 90% of copper with varying amounts of aluminium and nickel revealed one of the first recorded instances of " precipitation hardening " in copper-rich alloys.

Outside his college work Read was interested chiefly in the Volunteers and the Territorials, in which he held a commission for more than 25 years, receiving the Territorial Decoration. During the last war he was away from College on military service. After the war, the University of Wales and the College at Cardiff, both young institutions, were faced with serious problems, and Read sacrificed his personal inclination to resume his researches and applied himself energetically to University affairs. He brought to bear on academic matters an administrative outlook which was of inestimable value. His work was recognised by the University by conferring on him the degree of D.Sc. *honoris causa*, and the title of Emeritus Professor.

At first contact, Read was a little awe-inspiring to students entering his department, but he quickly won their confidence and respect, which ripened into affection under the influence of his kindly and genial disposition. To the students of the College as a whole he was a very good friend, always ready to champion their interests and to help on a good cause.

R. H. Greaves.
W. R. D. Jones.

JAMES McCONNELL SANDERS.

1875—1944

James McConnell Sanders went to Mexico in 1901, where he became Chief Chemist to the Mexican Customs, Head of the Research Department of the Government Medical Institute, and later Professor of Chemistry in the University of Mexico.

In 1915 he joined the Mexican Eagle Oil Co. as Chief Chemist to their refineries. In 1918 he returned to London and joined the Mexican Department of the Anglo-Saxon Petroleum Co. as chemical adviser. During the last three years before he retired he occupied the position of general consultant to the Shell Laboratories. In this position he was the more able to carry on with the study of the microchemistry of crude petroleum, a subject in which he had always taken a great interest, and which he had made his hobby.

He was a skilled manipulator and a good glass-blower and took a great pride in making his own apparatus, much of which was of an original type. His botanical knowledge was of great assistance to him in his microscopic examination of the residual dust obtained from various crude petroleums, from which he was able to draw conclusions as to the mother-substance of the petroleums which he examined.

He published a paper on " The Microscopical Examination of Crude Petroleum " in the *Journal* of the Institute of Petroleum in 1937.

He retired in 1939, but retirement for him meant the transfer of his laboratory to his own home, where he carried on until his death the work which was his much-loved hobby.

J. Kewley.

Some Aspects of the Chemistry of Nucleotides.

THE TILDEN LECTURE, DELIVERED BEFORE THE CHEMICAL SOCIETY ON FEBRUARY 18TH, 1943.

By J. MASSON GULLAND, M.A., D.Sc., Ph.D.

IN a lecture to the Society five years ago (Gulland, J., 1938, 1722) an attempt was made to review in outline the chemical knowledge which had at that time accumulated on the nucleic acids and related nucleotides. To-day it is far beyond the scope of a lecture to encompass the fields of research in which nucleotides play a part, since one of the striking developments of the intervening period has been the recognition of the widespread and fundamental intervention of nucleotides and polynucleotides (nucleic acids) into biological processes, as outlined below.*

The demonstration of the presence of deoxypentose nucleic acids in chromosomes and of pentose nucleic acids (nucleotides) in nucleoli, and the establishment of the existence of cycles of these acids in these tissues during cell divisions (Caspersson, *Skand. Arch. Physiol.*, Suppl., **1936**, **73**, No. 8; *J. Roy. Microscop. Soc.*, 1940, **60**, 8; Caspersson and Schultz, *Proc. Nat. Acad. Sci.*, 1940, **26**, 507) have given new conceptions of these processes and of the nature of the structures involved in them (Darlington, *Nature*, 1942, **149**, 66). The recognition of deoxypentose nucleic acids as highly anisometric molecules or micelles (Signer, Caspersson, and Hammarsten, *Nature*, 1938, **141**, 122; Greenstein and Jenrette, *J. Nat. Cancer Inst.*, 1940, **1**, 77, 91) with a nucleotide spacing of the same magnitude as that between the amino-acid side chains of an extended polypeptide has given rise to the belief that the nucleic acid and polypeptide molecules run lengthwise along the chromosomes, united through their phosphoric acid groups and basic side chains respectively (Astbury and Bell, *Nature*, 1938, **141**, 477; *Cold Spring Harbor Symposia Quant. Biol.*, 1938, **6**, 109; Schmidt, *Naturwiss.*, 1938, **26**, 413).

The viruses are consistently found to be nucleoproteins as each is isolated and characterised, the majority so far being derivatives of pentose nucleic acids (Hoagland, *Ann. Rev. Biochem.*, 1943, **12**, 615; Stanley, *ibid.*, 1940, **9**, 545). As nucleoproteins, they have a general chemical relationship with the chromosomes, and it is notable that they also share the unique faculty of self-perpetuation, and thus stand on the threshold between the living and the never-having-lived. This attribute is specially marked in the case of the viruses, some of which have been separated from the living cell as crystalline, homogeneous compounds, which can be submitted to investigation much as other chemical substances, but when re-introduced into other living cells produce symptoms of their presence identical with those induced before their original segregation.

Allied to the phenomena just described are the relationships of nucleic acids with tumour cells. Koller (*Nature*, 1943, **151**, 244) concludes from a cytological analysis of such cells that all chromosome abnormalities and the increased rate of division itself can be explained by the assumption of a qualitative change in the nucleic acid synthesis due to alteration in the heterochromatic region of the chromosomes. The ultra-violet absorption spectra of the cytoplasm of active tumour cells show accumulations of pentose nucleotides in amounts greater than those occurring in the corresponding healthy cells (Caspersson, Nyström, and Santesson, *Naturwiss.*, 1941, **29**, 29). Claude (*J. Exp. Med.*, 1935, **61**, 27, 41; 1937, **66**, 59; *Amer. J. Cancer*, 1937, **30**, 742; 1939, **37**, 59; *Science*, 1937, **85**, 294; 1938, **87**, 467; 1939, **90**, 213) has isolated from Rous chicken sarcoma extracts an extremely active, tumour-producing fraction which consists of a pentose-containing nucleoprotein in association with a phospholipoid component. He (*Proc. Soc. Exp. Biol. Med.*, 1938, **39**, 398; *Science*, 1940, **91**, 77; see also *Trans. New York Acad. Sci.*, 1942, **4**, 79) has also isolated from chick and mammalian embryos a similar fraction having no tumour-producing activity, and the question arises "whether the main constituents of the purified chicken tumour fraction represent inert elements existing also in normal cells; or whether the substance found in normal chick embryo tissue represents a precursor of the tumour principle, which could assume, under certain conditions, the self-perpetuating properties of the tumour agent." Similar phospholipoid–nucleoprotein complexes are found in embryonic tissue (Taylor, Sharp, Beard, and Beard, *J. Infect. Diseases*, 1942, **71**, 115), in embryonic and adult brain (Taylor, Sharp, and Woodhill, *Science*, 1943, **97**, 226), in adult liver (Claude, *Science*, 1940, **91**, 77; *Cold Spring Harbor Symposia Quant. Biol.*, 1941, **9**, 263), and in equine encephalomyelitis virus (Taylor, Sharp, Beard, Finkelstein, and Beard, *J. Infect. Diseases*, 1941, **69**, 224; Taylor, Sharp, Beard, and Beard, *ibid.*, 1943, **72**, 31).

Nucleic acids play essential rôles in embryonic development in several directions. Working with the sea-urchin egg, Brachet (*Compt. rend. Soc. Biol.*, 1931, **108**, 813, 1167; 1940, **133**, 90; *Arch. Biol.*, 1929, **39**, 677; 1933, **44**, 519; 1937, **48**, 529; 1940, **51**, 151) formulated the idea that during development the pentose nucleic acid which is present at first is changed into deoxypentose nucleic acid. Needham ("Biochemistry and Morphogenesis," 1942, p. 635, Cambridge Univ. Press) has summarised the ways in which nucleic acid metabolism may proceed, in four types of embryonic behaviour: (1) echinoderms, worms, molluscs, arthropods; simple transformation from stored pentose nucleic acid to deoxypentose nucleic acid: (2) amphibia; transformation, but synthesis of some deoxypentose nucleic acid: (3) teleostean and selachian fishes; store of purine and pyrimidine bases, followed by a synthesis of both forms of nucleic acid: (4) birds and reptiles; complete synthesis of both pentose and deoxypentose nucleic acids from non-cyclic precursors.

Caspersson and Schultz, using their ultra-violet absorption spectra technique, have obtained evidence of a

* It is intended that the two lectures should be read in conjunction,

relationship between acid metabolism and gene reproduction in the salivary chromosomes and the egg cytoplasm of *Drosophila melanogaster* (*Nature*, 1938, **142**, 294) and in cells where rapid division is occurring, including embryonic cells (*Nature*, 1939, **143**, 602; *Proc. Nat. Acad. Sci.*, 1940, **26**, 507).

Fischer (*Nature*, 1939, **144**, 113; *Chem. Prod.*, 1940, **3**, 79; *Acta Physiol. Scand.*, 1942, **3**, 54) has isolated from cow embryo a nucleoprotein, containing both pentose and deoxypentose nucleotides, which is highly effective in promoting the growth of explanted cells.

Damage to cells apparently evokes the production of pentose nucleotides: the cell-free filtrates from yeast cells damaged by ultra-violet light or mechanically have been shown by Loofbourow and his collaborators to contain a growth-promoting factor for yeast, and similar results were obtained with the cells of chick and mammalian embryos. The active factor, which is regarded as a physiological response to injury, is apparently associated with adenine (either alone or in conjunction with guanine), pentose and phosphorus, but not with uracil or deoxypentose, and is thus a pentose nucleotide derivative (Loofbourow and Heyroth, *Nature*, 1934, **133**, 909; Sperti, Loofbourow, and Dwyer, *ibid.*, 1937, **140**, 643; *Stud. Inst. Divi Thomae*, 1937, **1**, 163; Sperti, Loofbourow, and Lane, *Science*, 1937, **86**, 611; Loofbourow, Cook, and Stimson, *Nature*, 1938, **142**, 573; Loofbourow, Cook, Dwyer, and Hart, *ibid.*, 1939, **144**, 553; Loofbourow, Cueto, and Lane, *Arch. exp. Zellforsch.*, 1939, **22**, 607; Loofbourow, Dwyer, and Morgan, *Stud. Inst. Divi Thomae*, 1939, **2**, 137; Loofbourow and Dwyer, *Nature*, 1939, **143**, 725; 1940, **145**, 185; Loofbourow, *Biochem. J.*, 1942, **36**, 737; Cook, Cronin, Kreke, and Walsh, *Nature*, 1943, **152**, 474).

By means of ultra-violet photomicrography, Mitchell (*Brit. J. Exp. Path.*, 1942, **23**, 285, 296, 309) investigated the disturbance of nucleic acid metabolism produced by doses of X- and γ-radiations, and found that an increased ultra-violet absorption by cell cytoplasm, which occurs after irradiation, is caused by an accumulation of pentose nucleotides, which contain adenine and some other as yet unidentified light-absorbing groups.

Finally, it is well known that a pentose nucleotide, muscle adenylic acid (adenosine-5-phosphate), either as such or associated with other nucleotide-like compounds, occurs in cell cytoplasm as the essential co-enzymes of glycolysis, phosphoric acid transfer, hydrogen transfer and amino-acid oxidation.

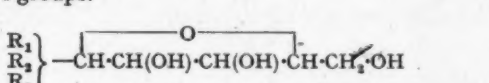
Underlying these spectacular biological advances and forming a unifying background, are the many, diverse chemical problems presented by the nucleotides and polynucleotides, and it is proposed to discuss some of these in this lecture.

The Synthesis of Nucleotides.

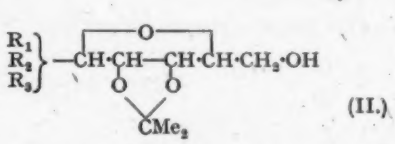
The synthesis of a nucleotide may be regarded conveniently as falling into two stages, the union of the nitrogenous base with the sugar by a glycosidic link to form a nucleoside, and the esterification of the sugar hydroxyl by the phosphoryl group. Methods for the synthesis of the purine nucleosides (Gulland and Story, J., 1928, 259) and the pyrimidine nucleosides (Hilbert, *J. Amer. Chem. Soc.*, 1937, **117**, 330; Hilbert and Rist, *J. Biol. Chem.*, 1937, **117**, 371) were described before the war, but the logical completion of these pieces of research, the synthesis of the four nucleosides themselves, has not been reached, partly no doubt owing to the difficulty of obtaining a sufficient quantity of the necessary sugar, ribofuranose, but also because of the general brake put on academic research by the war. Todd and his collaborators (J., 1943, 383, 386, 571, 574) have recently evolved a new mode of synthesising purine nucleosides, which offers greater scope in the production of analogues of the naturally occurring compounds than do the older syntheses.

Nevertheless, the second stage in the synthesis of nucleotides, the esterification by phosphoryl, has been studied in some detail by re-phosphorylation of the pentose nucleosides prepared by dephosphorylation of the natural nucleotides. These are therefore partial syntheses.

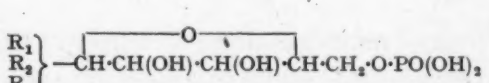
In proceeding along such lines the organic chemist thinks first in terms of protecting those hydroxyl groups of the sugar which are not to be esterified by radicals which can subsequently be removed by some gentle treatment. Adopting this procedure, Levene and Tipson (*J. Biol. Chem.*, 1934, **106**, 113; 1935, **111**, 313; 1937, **121**, 131) converted uridine (I, R_1), inosine (I, R_2) and adenosine (I, R_3) into the respective 5-phosphoesters (IV) by blocking the hydroxyls at C_2 and C_3 with the *iso*propylidene radical (II), phosphorylating the product with phosphorus oxychloride and pyridine (III), and removing the protective group by means of hot dilute acid; fortunately this reagent attacks the glycosidic and phosphoester links only slowly when the phosphoryl esterifies the hydroxyl at C_5, so that the *iso*propylidene radical can be hydrolysed preferentially. In a similar fashion these authors (*J. Biol. Chem.*, 1937, **121**, 131) and also Bredereck, Berger, and Ehrenberg (*Ber.*, 1940, **73**, 269) phosphorylated 2 : 3-diacetyl adenosine and obtained adenosine-5-phosphate by removing the acetyl groups.



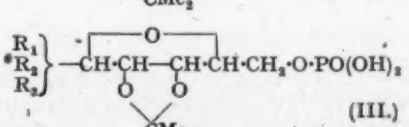
(I.)



(II.)

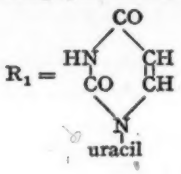


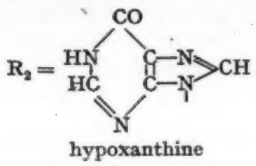
(IV.)

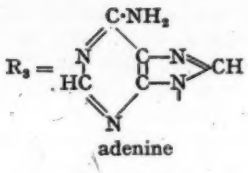


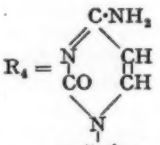
(III.)

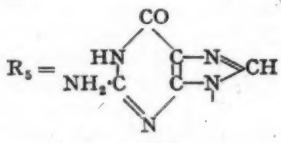
The following symbols are used throughout the lecture :—


uracil


hypoxanthine

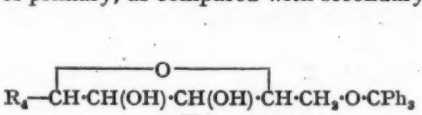

adenine

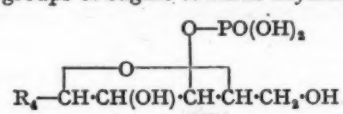

cytosine

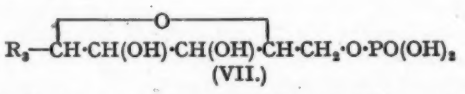

guanine

These nucleotides with phosphoryl at C_5 are, however, isomers of those obtained by hydrolysis of yeast ribonucleic acid, but Bredereck and his collaborators (*Ber.*, 1940, **73**, 269) obtained cytidylic acid (VI) itself, in the form of the brucine salt only, by blocking the hydroxyl at C_5 of cytidine with the trityl group (V), phosphorylating one of the free hydroxyls, and removing the protective trityl group. Esterification presumably occurred selectively at C_3, not at C_2, because the brucine salt isolated was identical with that of cytidylic acid obtained by alkaline hydrolysis of yeast ribonucleic acid; it is assumed that the phosphoryl group in natural cytidylic acid esterifies the hydroxyl at C_3.

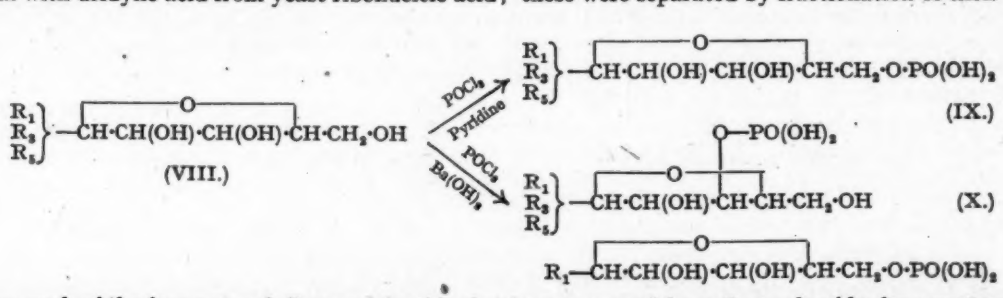
In all these syntheses the position of entry of the phosphoryl group is influenced by the presence of protecting groups previously introduced into the nucleoside, but in 1937 Jachimowicz (*Biochem. Z.*, 1937, **292**, 356) showed that phosphorylation of unprotected adenosine (I, R_3) itself with phosphorus oxychloride and pyridine yielded adenosine-5-phosphate (VII). This is to be expected, in view of the greater reactivity of the hydroxyls of primary, as compared with secondary, alcoholic groups of sugars towards acylating agents.


(V.)


(VI.)


(VII.)

Meanwhile it seemed possible that a mere change of the conditions of phosphorylation might determine the point of entry of the phosphoryl without reliance on protective groups, and this indeed proved to be the case. Almost simultaneously with the synthesis of cytidylic acid by Bredereck, Berger and Ehrenberg, a description was given by Gulland and Hobday (J., 1940, 746) of the phosphorylation of guanosine (VIII, R_3) and uridine (VIII, R_1), a purine and a pyrimidine nucleoside, with phosphorus oxychloride (*a*) in pyridine and (*b*) in saturated aqueous baryta. In pyridine both nucleosides yielded the 5-phospho-esters (IX); this was clear because the ester from uridine was identical with uridine-5-phosphate (Levene and Tipson, *loc. cit.*), and that from guanosine exhibited the greater stability of the phosphoryl towards acid hydrolysis which characterises the 5-phospho-esters of the pentose nucleosides as compared with the 3-phospho-esters. These results were thus in line with Jachimowicz's synthesis of adenosine-5-phosphate. On the other hand, phosphorylation of guanosine with phosphorus oxychloride and baryta yielded guanosine-3-phosphate (X) identical with guanylic acid prepared from yeast ribonucleic acid; adenosine (VIII, R_3) in the same circumstances behaved similarly and gave only adenosine-3-phosphate (X), but no 5-phosphate (Barker and Gulland, J., 1942, 231). Uridine under these conditions formed a mixture of uridine-5-phosphate (IX) and uridine-3-phosphate (X), identical with uridylic acid from yeast ribonucleic acid; these were separated by fractionation of their brucine salts.


(VIII.)

(IX.)

(X.)

Thus, each of the four natural ribose-nucleotides has been prepared from the nucleosides by a part-synthesis, together with three of the four isomeric 5-phosphorylated nucleotides, and methods have been established for introducing the phosphoryl radical at will into either of the two positions which it is known to occupy in Nature.

In speculations on the reasons for the divergent results of these two methods of phosphorylation, it seems possible that phosphorylation of the hydroxyl in the 5-position is simply the interaction of the more reactive and presumably more exposed primary alcoholic group with the acid chloride, the pyridine performing its usual functions in reactions of this type.

In the phosphorylations with baryta, however, the type of reaction may be different. It is evident from consideration of the dissociation constants of sugar hydroxyl groups (Hirsch and Schlags, *Z. physikal. Chem.*, 1929, **141**, *A*, 387; Stearn, *J. Physical Chem.*, 1931, **35**, 2226; Urban and Shaffer, *J. Biol. Chem.*, 1932, **94**, 697; Urban and Williams, *ibid.*, 1933, **100**, 237) and of the titration curves of the nucleotides (Levene and Simms, *ibid.*, 1925, **65**, 519) that partial dissociation of at least one of the sugar hydroxyls of the nucleosides occurs at 0° in saturated baryta solution, the conditions of the phosphorylation. It thus seems likely that phosphorylation of this salt is taking place, and in agreement with this, Fawaz and Zeile (*Z. physiol. Chem.*, 1940, **263**, 175) and Neuberg and Pollak (*Ber.*, 1910, **43**, 2060) failed to phosphorylate glucose with phosphorus oxychloride when barium or calcium hydroxide was replaced by calcium carbonate.

It might be thought that the primary alcoholic group would again be the more reactive, but in the relatively small number of cases where attempts have been made to ascertain which hydroxyls are concerned in salt formation, this is not always the case. Percival and his collaborators have shown that methylated sugars or their derivatives may be isolated when methylation is performed on the unstable complexes which are formed with potassium hydroxide by a variety of carbohydrates; from the constitutions of these methylated sugars it is deduced that in some cases primary, in others secondary, hydroxyls of the original sugars were the more active in complex formation with alkali (Percival, J., 1934, 1160; 1935, 648; Percival and Ritchie, J., 1936, 1765; Percival and Heddle, J., 1938, 1690). It seems, therefore, that the relative acidic strengths of the hydroxyl groups in the nucleosides are the factors which determine the position of the ester group in phosphorylation of the nucleotides in aqueous baryta, and that the reaction is analogous with a Schotten–Baumann acylation.

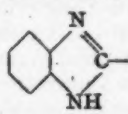
The Nature and Biogenesis of the Pentose of Nucleotides.

During the twenty years ending in 1912 there were many speculations as to the nature of the pentose in inosinic acid and the nucleic acid of yeast and pancreas. The ramifications of the story are complex, but *d*-xylose, *d*-lyxose and *dl*-arabinose were all proposed at various dates, on evidence which cannot always be lightly dismissed, although certainly not conclusive. In 1909, however, Levene and his collaborators isolated a crystalline sugar from inosine, prepared from inosinic acid, and declared this to be *d*(-)ribose. Immediately afterwards this claim was extended to the sugar of guanylic acid from liver, from pancreas and from yeast nucleic acid, and to the adenosine prepared from that acid.

For the past thirty years this conclusion—*d*(-)ribose—has been accepted. There has indeed been a tendency to assume, with no proof, that the sugar of all pentose-containing nucleotides is necessarily *d*-ribose, although examination has been insufficient to permit a statement that this is, or is not, the case; it is of interest in this connection that Loring (*J. Biol. Chem.*, 1939, **130**, 251) has recorded the isolation from the pentose nucleic acid of tobacco mosaic virus of an isomeric uridylic acid, differing from the normal acid in the physical properties of its brucine salt; this may possibly be due to the presence of a different pentose in the virus nucleotide. A review (Barker and Gulland, J., 1943, 625) of the literature, however, suggested that it was desirable to confirm Levene's conclusions, particularly because the nucleotides are the only natural occurrence of the sugar ribose. The pentose nucleic acid of yeast was selected for examination in the first instance, and the sugar components were oxidised to the aldonic acids (XI) and identified as the phenylhydrazides (XII) and as the benziminazole derivatives (XIII) (Moore and Link, *J. Biol. Chem.*, 1940, **133**, 293), prepared by condensation with *o*-phenylenediamine. The results obtained confirm Levene's claim that the sugar of the four nucleotides of yeast nucleic acid is *d*(-)ribose (XIV), and the examination is being extended to the pentoses of other nucleotides and nucleic acids. One point arising from this work is worth mention. *l*-Lyxose (XIX) was isolated in small amount, in the form of the benziminazole derivative, from the hydrolysis products of a commercial sample of yeast nucleic acid, and it is believed that this is the first time that this sugar has been identified in naturally occurring compounds. It is possible that it is not so rare, but that the difficulty of crystallising it or its derivatives has hitherto prevented its recognition; if so, then the benziminazole technique should prove useful, since the separation of the sugar in this way presents little difficulty.

$$CO_2H\cdot CH(OH)\cdot CH(OH)\cdot CH(OH)\cdot CH_2\cdot OH \quad (XI.)$$

$$NHPh\cdot NH\cdot CO\cdot CH(OH)\cdot CH(OH)\cdot CH(OH)\cdot CH_2\cdot OH \quad (XII.)$$



(XIII.)

This confirmation of the nature of the sugar re-opens the question of its biogenesis and the form in which it occurs in the nucleotides.

As part of a wider theory of the biogenesis of naturally occurring sugars, Robinson (*Nature*, 1927, **120**, 44, 656) put forward the suggestion that the nucleotides of yeast nucleic acid are really 3-phospho-derivatives of the common sugar *d*-xylose (XV) and that on dephosphorylation (by chemical hydrolysis) Walden inversion

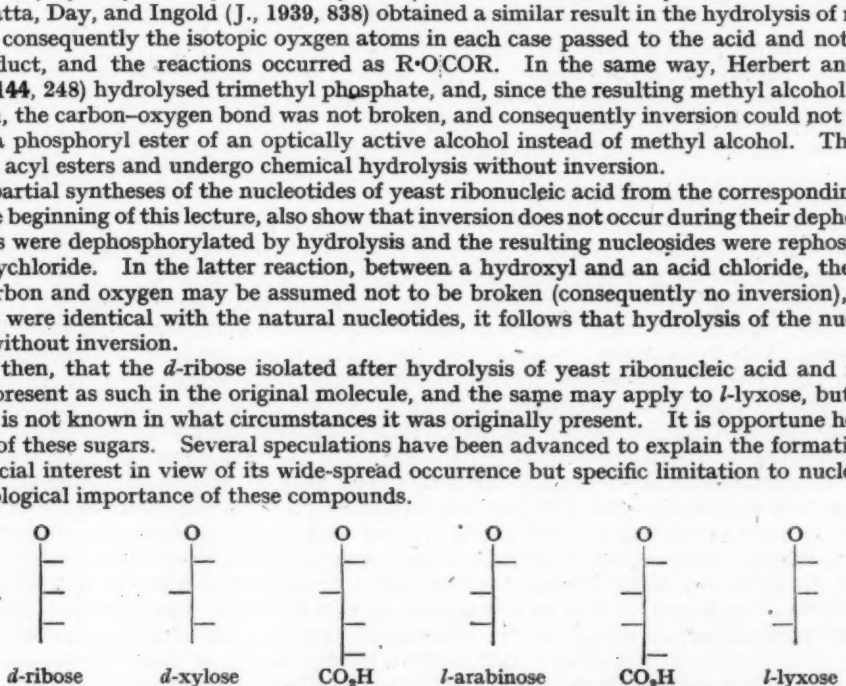
takes place at C_3, yielding the nucleosides as ribosides. He drew support for this suggestion from the possible analogy with the inversion observed (Phillips, J., 1925, **127**, 2552; Kenyon, Phillips, and Taylor, J., 1933, 173) when sulphonic esters are hydrolysed. Levene (*Nature*, 1927, **120**, 620) did not accept Robinson's suggestion. There the matter apparently rested, probably because there was at that time insufficient evidence to proceed further. This has now accumulated from three standpoints and unfortunately does not support the hypothesis, at any rate in its original form.

First, knowledge of the mechanism of the Walden inversion in the hydrolysis of carbohydrate esters has been greatly extended since 1927, and it is known that when inversion occurs during the hydrolysis of the ester, *e.g.*, toluenesulphonyl esters, it is accompanied practically without exception by the formation of an anhydro-sugar, in which is involved an adjacent hydroxyl originally in the *trans*-position to the ester group. Further hydrolysis of this anhydro-ring to reconstitute a fully hydroxylated sugar results in inversion at one or other of its two carbon atoms; the sugars thus formed have the hydroxyl groups once more in the *trans*-position relative to each other. It is evident, therefore, that *d*-ribose, with three *cis*-hydroxyls, is unlikely to be formed by chemical hydrolysis of an ester of *d*-xylose.

Second, an essential condition for the occurrence of a Walden inversion is that a true replacement should occur, that is, that the bond must be broken which joins the asymmetric atom to the group being displaced. This always happens when, for example, a halogen is being replaced by a hydroxyl or an amino-group, but when an ester group is replaced by hydroxyl this condition may, or may not, be fulfilled. Thus, inversion is rare in the hydrolysis of acyl esters, which are therefore regarded as undergoing fission as RO·COR, but is common with sulphonyl esters, which are therefore hydrolysed as $RO{\cdot}SO_2R$. Robinson's suggestion, based on a supposed similarity between phosphoric esters and sulphonic esters, would imply fission of the phosphoric esters as $RO{\cdot}PO(OH)_2$ in order to allow inversion. The most clean-cut evidence in the mechanism of hydrolysis of esters is by the use of water containing an excess of heavy oxygen. Polanyi and Szabo (*Trans. Faraday Soc.*, 1934, **30**, 508) hydrolysed amyl acetate by this means and obtained amyl alcohol containing no heavy oxygen, and Datta, Day, and Ingold (J., 1939, 838) obtained a similar result in the hydrolysis of methyl hydrogen succinate; consequently the isotopic oyxgen atoms in each case passed to the acid and not the alcoholic hydrolysis product, and the reactions occurred as R·O·COR. In the same way, Herbert and Blumenthal (*Nature*, 1939, **144**, 248) hydrolysed trimethyl phosphate, and, since the resulting methyl alcohol was free from isotopic oxygen, the carbon–oxygen bond was not broken, and consequently inversion could not have occurred in the case of a phosphoryl ester of an optically active alcohol instead of methyl alcohol. Thus phosphoric esters resemble acyl esters and undergo chemical hydrolysis without inversion.

Third, the partial syntheses of the nucleotides of yeast ribonucleic acid from the corresponding nucleotides, described at the beginning of this lecture, also show that inversion does not occur during their dephosphorylation. The nucleotides were dephosphorylated by hydrolysis and the resulting nucleosides were rephosphorylated by phosphorus oxychloride. In the latter reaction, between a hydroxyl and an acid chloride, the link between asymmetric carbon and oxygen may be assumed not to be broken (consequently no inversion), and since the synthetic acids were identical with the natural nucleotides, it follows that hydrolysis of the nucleotides must have occured without inversion.

It is clear, then, that the *d*-ribose isolated after hydrolysis of yeast ribonucleic acid and its component nucleotides is present as such in the original molecule, and the same may apply to *l*-lyxose, but with less certainty, since it is not known in what circumstances it was originally present. It is opportune here to consider the biogenesis of these sugars. Several speculations have been advanced to explain the formation of *d*-ribose, which is of special interest in view of its wide-spread occurrence but specific limitation to nucleotides, and of the marked biological importance of these compounds.

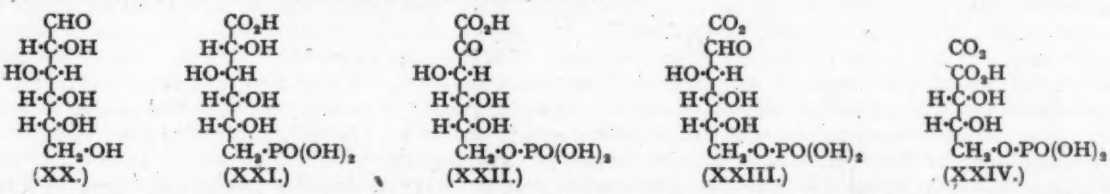


d-ribose (XIV.) — *d*-xylose (XV.) — *d*-glucuronic acid (XVI.) — *l*-arabinose (XVII.) — *d*-galacturonic acid (XVIII.) — *l*-lyxose (XIX.)

It has long been suggested that the naturally occurring pentoses have their origin in the hexuronic acids, which decompose into pentose and carbon dioxide (see, for example, Hirst, J., 1942, 70; Hirst and Jones, *Chem. and Ind.*, 1937, **56**, 724). This reaction has not been demonstrated in an isolated enzyme system, although Salkowski and Neuberg (*Z. physiol. Chem.*, 1902, **36**, 261) have shown that glucuronic acid is decarboxylated to *d*-xylose by bacteria isolated from putrefying flesh. That the reaction is of wider significance, however, is highly probable from the frequent occurrence of xylose (XV) of the *dextro*-series, arising from *d*-glucuronic acid (XVI), but of arabinose (XVII) of the *lævo*-series which would be formed from *d*-galacturonic acid (XVIII); these uronic acids occur naturally, and the related hexoses are frequently accompanied by the corresponding pentoses. If all pentoses are formed in this straightforward manner, the precursor of *d*-ribose would be *d*-alluronic or *d*-taluronic acid, and of *l*-lyxose would be *l*-mannuronic or *d*-guluronic acid, none of

which is found in Nature. Consequently, if this theory of the formation of pentoses is to be generally accepted, it is necessary to assume that Walden inversion occurs in the formation of *d*-ribose and *l*-lyxose, at C_3 of *d*-glucuronic and *d*-galacturonic acids respectively. In this connection it may be relevant to note that so far ribose is known to occur naturally only when phosphorylated at C_3 (VI and X) (guanylic, adenylic, cytidylic and uridylic acids of pentose nucleic acids) or at C_5 [muscle adenylic acid (VII) and the adenine co-enzymes]; the two exceptions, uric acid riboside (Newton and Benedict, *J. Biol. Chem.*, 1922, **54**, 595; Falconer and Gulland, J., 1939, 1369) and crotonoside (*iso*guanine riboside; Cherbuliez and Bernhard, *Helv. Chim. Acta*, 1932, **15**, 464, 978; Spies and Drake, *J. Amer. Chem. Soc.*, 1935, **57**, 774; Spies, *ibid.*, 1939, **61**, 350; Falconer, Gulland, and Story, J., 1939, 1784), can probably be regarded as dephosphorylated nucleotides. A possible hypothesis might then be that inversion is a necessary consequence of the enzymic phosphorylation at C_3 of these uronic acids. In that case the formation of muscle adenylic acid and its derivatives would be secondary to the production of ribose-3-phosphate, and the enzymic mechanism for this stage from adenosine, is well established (Ostern and Terszakoweć, *Z. physiol. Chem.*, 1937, **250**, 155, and subsequent papers), whereas the enzymic phosphorylation of ribose nucleosides at C_3 is not known. Against this hypothesis is the fact that the conversion of 3-phosphoglyceric acid into 2-phosphoglyceric acid by phosphoglyceromutase apparently proceeds without inversion (Kiessling and Schuster, *Ber.*, 1938, **71**, 123, footnote 9); other examples of enzymic phosphorylation of the hydroxyl at an asymmetric carbon atom are lacking.

A route to *d*-ribose from glucose (XX), which has many attractions, has been advanced by Dickens (*Nature*, 1936, **138**, 1057; *Biochem. J.*, 1938, **32**, 1626, 1645), who assumes that the first stage is esterification to glucose-6-phosphate. An enzyme prepared from Lebedew yeast maceration fluid converts this ester by way of phosphogluconic acid (XXI) and 2-ketophosphohexonic acid (XXII) into pentose-5-phosphoric acid (XXIII) and thence by further oxidation into phosphorylated hydroxy-carboxylic acids of lower carbon content (XXIV). The pentose-phosphoric acid has not as yet been isolated as a pure compound, but the presence of pentose has been indubitably demonstrated. In this series of reactions it might be anticipated that the pentose formed would have been *d*-arabinose-5-phosphoric acid (XXIII), but in concurrent experiments it was demonstrated that *d*-ribose-5-phosphate not only underwent further oxidation by the enzyme, but was also fermented considerably more readily than *d*-arabinose-5-phosphoric acid or *d*-xylose-5-phosphoric acid. The theory was therefore advanced that *d*-ribose-5-phosphoric acid may originate from hexoses by a process of phosphorylation, oxidation and decarboxylation, inversion occurring at C_3 of glucose. If this mechanism is accepted, a possible mode of formation of the sugar moiety of adenylic acid and its derivatives seems clear, and the 3-phosphonucleotides of pentose nucleic acids might be secondary products arising through an enzymic phosphorylation which has not as yet been demonstrated satisfactorily *in vitro*.



Other hypotheses involve the aldol condensation of lower sugars, as, for example, the condensation of glycollaldehyde with *d*-glyceraldehyde phosphate, or with dihydroxyacetone phosphate, followed by an immediate rearrangement to the aldose series, postulated by Gardner (*J. Org. Chem.*, 1943, **8**, 111).

It is clear that the problem of the biogenesis of ribose and of the nucleotides can gain little more from speculation and can be advanced only by experimental investigation.

*The Structure of Nucleic Acids.**

No matter from what sources nucleic acids are isolated, and whether animal or vegetable, present knowledge allows them to be classified as belonging to either of two main types, pentose nucleic acids and deoxypentose nucleic acids. This conclusion is based on the structures of the four nucleotides into which each type of acid may be broken by suitable means, the former type by alkaline hydrolysis, the latter by enzymic fission (see Gulland, J., 1938, 1722).

Until relatively recently it was generally assumed that the molecule of each type of acid consists of a tetranucleotide, that is, a molecule formed by union of one of each of the four nucleotides. The reasons for this belief arose chiefly from five causes (see Gulland, J., 1938, 1728, for a more detailed account). First, it was often assumed that the major features of the structures of the acids of both types were the same, and that in consequence conclusions reached from experiments on one acid could be applied directly to considerations of the other type. Second, Jones's ("Nucleic Acids," 1920, p. 40, Longmans Green and Co., London) quantitative hydrolysis of the pentose nucleic acid of yeast showed that it contained equimolecular amounts of purine and pyrimidine nucleotides, and this, in conjunction with the isolation of the two purine and two pyrimidine bases themselves after drastic degradation, apparently clinched the tetranucleotide hypothesis. Third, and this was a powerful factor, Myrbäck and Jorpes (*Z. physiol. Chem.*, 1935, **237**, 159) recorded for the pentose nucleic acid of yeast a molecular weight corresponding roughly to that of a single tetranucleotide. Fourth,

* Space restrictions prohibit a discussion in greater detail than is given here.

data on the titration of both types of acid accumulated independently by Hammarsten, Levene, Makino, Bredereck, and ourselves showed that the acids exhibited approximately either 4 or 5 phosphoric acid dissociations. The presence of five acidic groups, believed to be four primary and one secondary, could only be explained on the basis of a tetranucleotide having an open-chain structure, and this could have arisen by hydrolysis of the other alternative, a cyclic tetranucleotide with four primary acidic dissociations, each deriving from a doubly esterified phosphoryl radical; the latter conception not only fell into line with the results of the molecular weight determinations of Myrbäck and Jorpes, but also agreed with the results of enzymic fission. Fifth, specific enzymes, phosphomonoesterase and phosphodiesterase, were shown by Takahashi (*J. Biochem. Japan*, 1932, **16**, 463) and by Gulland and Jackson (J., 1938, 1492) to fail to cause dephosphorylation of the pentose nucleic acid of yeast when acting separately, whereas a mixture of the enzymes did so; such results favoured the cyclic tetranucleotide.

Despite this evidence pointing to the simple tetranucleotide, it is now clear that the true molecular sizes of both types of nucleic acid are larger, in some cases much larger, and that they vary considerably with the type and source of the acid and also with the method of isolation and treatment during purification; alkali is specially effective in reducing molecular size, undoubtedly because it causes hydrolysis.

Considering deoxypentose nucleic acid first, Signer, Caspersson, and Hammarsten (*Nature*, 1938, **141**, 122) found the molecular weight of the sodium salt of the acid from thymus to be between 500,000 and 1 million from studies of viscosity and double refraction of flow, that is, about 1500 to 3000 nucleotides, since the average weight of a nucleotide is about 330; the length of these particles was 300 times their width. Astbury and Bell (*ibid.*, p. 747) confirmed this size as being approximately 2000 nucleotides from *X*-ray studies of stretched films of the sodium salt. Schmidt, Pickels, and Levene (*J. Biol. Chem.*, 1939, **127**, 251) obtained sedimentation data showing that the nucleic acid of thymus, prepared by different methods, was a mixture of substances ranging in molecular weight from about 1500 to over 1 million, the molecular size being dependent on the mode of preparation. Tennent and Vilbrandt (*J. Amer. Chem. Soc.*, 1943, **65**, 424), also using the ultracentrifuge, found a molecular weight of 500,000 for the sodium salt of the acid from thymus. Characteristic properties of these large molecules are the gel-formation and streaming birefringence shown by aqueous solutions of the sodium salts; the particles are highly asymmetric in shape, very long compared with their width.

On the other hand, using a drastic alkaline method of purification, Bredereck and Jochmann (*Ber.*, 1942, **75**, 395) obtained from the deoxypentose nucleic acid of spleen an acid which may have been a true tetranucleotide; it was soluble in water, unlike the more complex material, had approximately the correct analytical composition and molecular weight for a compound composed of one molecule of each of the nucleotides, had five acidic dissociations, and did not exhibit streaming birefringence or form a gel.

Turning next to the pentose nucleic acids, Loring (*J. Biol. Chem.*, 1939, **128**, Sci. Proc. **33**, 61) used the Northrop–Anson diffusion method of analysis and found molecular sizes of 116 nucleotides for the acid of tobacco mosaic virus and 56—88 nucleotides for the acid of yeast. Later Cohen and Stanley (*J. Biol. Chem.*, 1942, **144**, 589) carried out a detailed study of the physical properties of the acid of tobacco mosaic virus, isolated by heat denaturation of the virus protein and thus avoiding the use of alkali; it is therefore possible that this material was, or approached closely to, the true native nucleic acid of the virus. They studied electrophoresis, partial specific volume, diffusion, sedimentation, viscosity, electron microscopy, osmotic pressure, and optical properties. The freshly isolated acid had an approximate particle weight of 300,000 and was highly asymmetric in shape with axial ratio about 60; it decomposed spontaneously to form still asymmetric, birefringent particles with molecular weight about 61,000. Cold alkali converted each of these particles into molecules with a weight of 15,000 and a length–width ratio of 10. Cohen and Stanley state that there is at present no evidence that a unit smaller than *M* 15,000 and larger than a nucleotide exists as a fundamental unit of the virus nucleic acid. Fischer, Böttger, and Lehmann-Echternacht (*Z. physiol. Chem.*, 1941, **271**, 246) found the molecular weight of the pentose nucleic acid of yeast to be 10,350 by a dialysis method. According to our own measurements (Fletcher, Gulland, Jordan, and Dibben, J., 1944, 30), the molecular weight of the acid from yeast ranges between 10,280 (32 nucleotides) and 23,250 (70 nucleotides), depending on the commercial source from which the samples were originally derived and hence possibly on their treatment during extraction.

Yeast ribonucleic acid readily suffers complete fission to nucleotides by alkaline hydrolysis, but by regulating the conditions so as to avoid this full decomposition, Bredereck and Hoepfner (*Ber.*, 1942, **75**, 1086) obtained what they considered to be the tetranucleotide of this acid. This material had the properties which might be expected, *viz.*, solubility in water, a molecular weight of 1177 (calc., 1304), the correct analytical composition of a molecule built up of one of each of the four nucleotides, and five phosphoric acid dissociations. In addition, this so-styled tetranucleotide was stated to undergo smooth deamination to a deaminated tetranucleotide, which was still pentabasic when titrated to the phenolphthalein end-point, and which yielded xanthine (from guanine) on hydrolysis. Unfortunately for the claim of Bredereck and Hoepfner to have isolated a tetranucleotide unit, it has been shown (Fletcher, Gulland, and Jordan, J., 1944, 33) that theory demands that this deaminated tetranucleotide should have been hexabasic, since the hydroxyl group of xanthine titrates over the range pH 5—8. Further, a careful repetition by us of Bredereck and Hoepfner's hydrolysis experiment yielded material of higher average molecular weight and greater acidity than expected, and it seems necessary to stress our present opinion that both Bredereck and Hoepfner's and our material were mixtures of indefinite composition and that there is no evidence on these grounds for the existence of a pentose tetranucleotide.

The question of molecular size of nucleic acids, true molecular size in contrast to micellar size, is complicated

by a reversible aggregation or polymerisation observed in the case of deoxypentose nucleic acids. Indeed Pedersen (quoted by Svedberg and Pedersen, "The Ultracentrifuge," 1940, p. 443, Oxford Univ. Press) found that the sodium salt of this acid was not monodisperse and had an average molecular weight of the order of 200,000 in dilute solution, but the sedimentation constant was highly dependent on the concentration. With these colloidal aggregates the particle size may be dependent not only on the concentration of the nucleic acid, but also on the concentration of the buffer solution in which it is dispersed. The spontaneous fall in molecular weight of tobacco mosaic virus nucleic acid mentioned above may be a micellar disaggregation of an analogous type.

It is appropriate here to mention the work of Greenstein and Jenrette (*J. Nat. Cancer Inst.*, 1940, **1**, 77). Considerable structural viscosity and double refraction of flow were marked characteristics of all samples of deoxypentose nucleic acid examined from various sources, although the magnitudes of these properties varied appreciably even when these samples were prepared from the same source by the same method. The addition of certain salts to solutions of the sodium salt of this acid resulted in a parallel decrease of viscosity and intensity of streaming birefringence, and at a sufficiently high concentration of the added salt neither property could any longer be observed; guanidinium was the most effective cation, and iodide the most efficient anion. Both birefringence and viscosity were almost completely restored to the nucleic acid by removal of the added salt, either by dialysis or by precipitating the nucleic acid. Greenstein and Jenrette attributed these phenomena to a depolymerisation of large to small particles, which then repolymerised on removal of the salt. No molecular weights, however, were recorded for these so-called smaller particles, and consequently it is difficult to place an exact interpretation on the results, for which alternative explanations are possible.

Numerous investigators have studied the action on both types of nucleic acid of those enzymes which bring about hydrolytic fission to molecules greater than nucleotides without any simultaneous loss of groups or radicals (Dubos, *Science*, 1937, **85**, 549; Dubos and Thompson, *J. Biol. Chem.*, 1938, **124**, 501; Schmidt and Levene, *Science*, 1938, **88**, 172; Schmidt, Pickels, and Levene, *J. Biol. Chem.*, 1939, **127**, 251; Schmidt and Levene, *ibid.*, 1938, **126**, 423; Kunitz, *Science*, 1939, **90**, 112; *J. Gen. Physiol.*, 1940, **24**, 15; Allen and Eiler, *J. Biol. Chem.*, 1941, **137**, 757; Eiler and Allen, *Proc. Soc. Exp. Biol. Med.*, 1941, **46**, 436; Greenstein and Jenrette, *J. Nat. Cancer Inst.*, 1941, **1**, 845; **2**, 301; Fischer, Böttger, and Lehmann-Echternacht, *Z. physiol. Chem.*, 1941, **271**, 246; *J. pr. Chem.*, 1941, **158**, 79; Bolomey and Allen, *J. Biol. Chem.*, 1942, **144**, 113).

For each type of acid there appears to be a specific depolymerising enzyme (known at present as ribonuclease, and deoxyribonuclease or thymonucleodepolymerase respectively), wide-spread in both animal and plant tissues, which rapidly reduces the molecular size, and with it the viscosity and streaming birefringence when these are evident. These enzymes do not cause dephosphorylation. They are highly specific for the particular nucleic acid type, and act neither on a wide range of natural synthetic substances which have been offered as substrates, nor on the other type of nucleic acid; Bredereck and Müller (*Ber.*, 1939, **72**, 1429) believe, however, that one enzyme alone exists and that it acts on both acids. This high specificity has given rise to the suggestion that the natures of the linkages of the tetranucleotides in the two types of nucleic acid are essentially different. Whether in fact they are, or are not, different, a conclusion based on this line of argument alone seems to be scarcely tenable in view of the extremely selective specificity of various enzymes.

The final products in the case of the deoxypentose nucleic acid are apparently tetranucleotides; Fischer and his collaborators showed that the molecular size, measured by a method admittedly incapable of high accuracy, lies between 3·6 and 5·3 mononucleotides and that there is an increase in titratable acidity of one group for every four atoms of phosphorus, a change in the total number of acid groups from four to five. It is not clear, however, whether these tetranucleotides are the same as the material obtained through alkaline hydrolysis by Bredereck and Jochmann.

In the case of the pentose nucleic acid of yeast, Fischer and his collaborators recorded that the products of depolymerisation were dinucleotides with an average molecular weight of 690, whereas Allen and Eiler stated that they were tetranucleotides with an increase in titratable acidity of one group for every four atoms of phosphorus. Further, Allen and Eiler showed that this group is a secondary phosphoryl dissociation because of its titration range, and this implies that the enzymic hydrolysis occurs at a phosphoryl which already has one acidic dissociation. These divergences need further investigation, although it seems possible that Fischer's dinucleotides might be the results of further hydrolysis of the tetranucleotide.

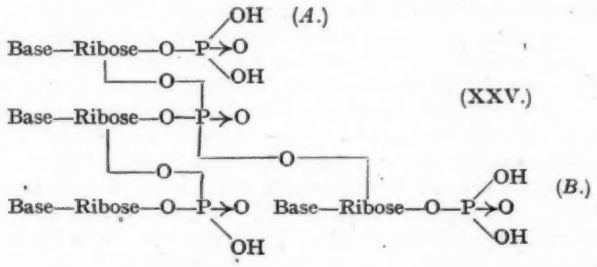
It is thus universally agreed that the molecules of nucleic acids are composed of large numbers of nucleotides, but it must be confessed that remarkably little is known with certainty of the inner structures of these molecules, the modes of combination of these nucleotides. For example, there is as yet no evidence as to whether the nucleic acids of each type are, or are not, identical in structure amongst themselves, irrespective of their source, or even whether there is any general nucleic acid pattern common to all those nucleic acids which belong to one type but are derived from different sources.

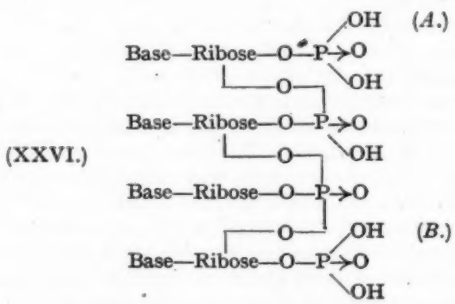
From time to time suggestions have been made that the molecules of deoxypentose nucleic acids and yeast ribonucleic acid are polytetranucleotides, with an assumption that each tetranucleotide unit of the polymer is identical with its neighbours, as regards both the sequence of its component nucleotides and its uniform content of one molecule of each of these; this postulate, however, has not been made for the pentose acid of tobacco mosaic virus, the most fully investigated of the virus acids. It should be realised that the conception of a molecule composed of polymerised tetranucleotides has grown from a mental superposition of the later demonstrations of high molecular weights on to the older ideas of a simple molecule containing one of each of the four

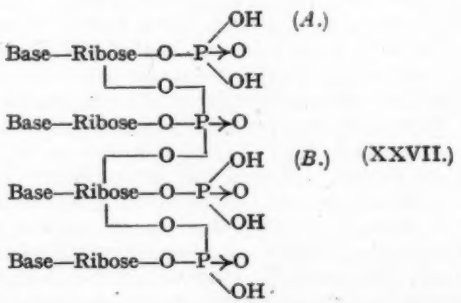
appropriate nucleotides; had the true molecular sizes been realised earlier, it is doubtful whether the conception would have gained such firm hold as is apparently the case.

At present the facts tend to favour the hypothesis of molecules composed of polymerised tetranucleotides in the cases of yeast ribonucleic acid and some examples, at any rate, of deoxypentose nucleic acids, but sound evidence is non-existent either to support the existence of a uniform tetranucleotide composed of one of each of the appropriate nucleotides or to indicate a regular sequence of nucleotides. Turning to pentose nucleic acids in general, there are contrary indications to a uniform content of the four nucleotides. For example, Graff and Maculla (*J. Biol. Chem.*, **1935**, **110**, 71) showed that in the ribose acid from yeast the percentage of purines is almost that required for equimolecular proportions of the four nucleotides, but in tobacco mosaic virus nucleic acid Loring (*ibid.*, **1939**, **130**, 251) found a 20% deficiency of purines, and in the allonucleic acid of pancreas the guanine nucleotides predominate (see Gulland, J., **1938**, 1722).

Nevertheless, the conception of two uniform tetranucleotides for all nucleic acids, one containing pentose, the other deoxypentose, each containing one of each of the four appropriate nucleotides, can form a practical working hypothesis, which is helpful in the interpretation of experimental results, provided its limitations are fully realised.







In support of this hypothesis, in so far as it concerns yeast ribonucleic acid, it is appropriate to mention the results of work (Fletcher, Gulland and Jordan, J., **1944**, **33**) on electrometric titrations of samples of this acid having molecular weights of approximately 10,000, 15,000, and 22,000. Comparison of the experimental curves with those constructed theoretically from a knowledge of the dissociation constants of the groups present showed that for each four atoms of phosphorus there were four acidic dissociations of phosphoryl radicals, three primary and one secondary. This result was fully confirmed by similar titration of the deaminated nucleic acid, which can be prepared under particular conditions without lowering of molecular weight. Basing these observations on the assumption of an acid composed of polymerised, uniform, hypothetical tetranucleotides containing one molecule of each nucleotide, it follows that one phosphoryl radical per tetranucleotide must be triply linked and be neutral; it is believed that this is that radical which forms part of uridylic acid. Two dissociations must be primary, associated with a phosphoryl group which is doubly linked to other radicals; and one phosphoryl group can be only singly linked to a ribose radical and must exhibit a primary and a secondary dissociation. Possible formulæ for the tetranucleotide which are in agreement with the observed facts are (XXV), (XXVI) and (XXVII); polymerisation to form the polytetranucleotide would occur either through the group (*A*) or (*B*), the other member of this pair remaining singly linked.

Mild alkaline hydrolysis of the nucleic acid liberates secondary phosphoryl dissociations, and this is confirmed by the hydrolysis of the deaminated acid in boiling water, one secondary phosphoryl dissociation then being liberated for each tetranucleotide; the product has a molecular weight of roughly the correct order for a deaminated tetranucleotide and does not undergo further hydrolysis under the conditions of the experiment. Consequently, when the tetranucleotide of the acid comes to be isolated, it should exhibit three primary and two secondary phosphoryl dissociations, not four and one respectively as always postulated hitherto. It also shows that the fission of the acid into tetranucleotides by chemical means hydrolyses a link involving a secondary acidic group of a phosphoryl radical; the same is true for the enzymic fission by ribonuclease, but it is unknown whether the resulting tetranucleotides are identical and the same phosphoryl radical is concerned in both kinds of hydrolysis.

A further point arising from this work is worth mention. Examination of the analyses in the literature

over many years shows that there is a marked tendency for the figures for phosphorus to be low. On the basis of a polymer of tetranucleotides constituted as hitherto believed, namely, tetranucleotides with four primary and one secondary dissociation united by means of the secondary acidic group, a detachment of phosphorus could only occur by disruption of the nucleic acid molecule, except for the removal of the singly linked terminal radical. No such disruption had apparently taken place, and the extent of deficiency of phosphorus was often greater than could be accounted for by removal of the terminal, singly linked phosphoryl group. On the basis of a polymer made up of the tetranucleotide now proposed, however, it is possible for up to one-quarter of the total phosphorus, as theoretically required, to be absent without affecting the main structure of the polymer, since each tetranucleotide bears one phosphoryl group which is singly linked and plays no part in maintaining the skeletal structure.

I wish to acknowledge my indebtedness to my colleagues Dr. G. R. Barker and Mr. D. O. Jordan, M.Sc., with whom I have discussed many points in this lecture.

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56. *Lactones of Mannosaccharic Acid. Part I. 2 : 5-Dimethyl Δ^4-Mannosaccharo-3 : 6-lactone 1-Methyl Ester, an Analogue of Ascorbic Acid.*

By W. N. Haworth, (Miss) D. Heslop, (Miss) E. Salt, and F. Smith.

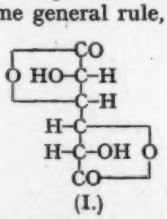
Mannosaccharodilactone, prepared from either mannose or α-methylmannoside by oxidation with nitric acid, reduces Fehling's solution, a property not displayed by simple monolactones of either the γ- or the δ-series. This phenomenon was observed by Kiliani (*Ber.*, 1887, **20**, 2710) and by Fischer (*Ber.*, 1891, **24**, 539), but a satisfactory explanation based on experimental facts has never been put forward. Mannosaccharodilactone only displays reducing properties in alkaline solution or after treatment with alkaline reagents. The substance which is believed to be responsible for these reducing properties shows in alkaline solution an intense selective absorption band at λ 2630 A., moving to λ 2290 A. upon acidification. This paper deals with the methylation of mannosaccharodilactone, a process which appears to isomerise the dilactone in the same way as do alkaline reagents, and which also stabilises the isomeric form of the dilactone as a methyl derivative.

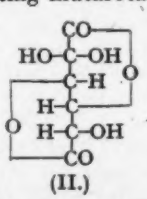
Treatment of mannosaccharodilactone with diazomethane, with silver oxide and methyl iodide, and with diazomethane, followed by silver oxide and methyl iodide, affords mainly 2 : 5-*dimethyl* Δ^4-*mannosaccharo*-3 : 6-*lactone* 1-*methyl ester* (VI), a substance showing a strong band at λ 2290 A. Methylation of mannosaccharodilactone is not a simple process, because in addition to (VI) there are produced 6-carbomethoxy-3-methoxy-α-pyrone, methyl dimethyl *meso*tartrate, and a trimethyl mannosaccharolactone methyl ester.

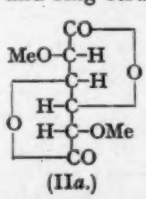
Ozonisation of (VI) gives oxalic acid and an aldehydic acid, a monomethyl erythuronic acid (VII). Oxidation of the latter with bromine yields the new 3-*hydroxy*-2-*methoxy*-1-*erythrosuccinic acid* (VIII), the configuration of which has been established; the acid (VIII) was characterised as a crystalline *diamide* and a crystalline *bismethylamide*. These facts prove the structure (VI) assigned to the methylated unsaturated lactone methyl ester. Confirmation of this conclusion followed from the fact that reduction of (VI) gives the 4-*deoxy*-derivative (XIV), the crystalline *diamide* (XV) of which shows a negative Weerman test for α-hydroxy-amides.

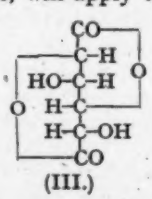
It is suggested that the reducing substance produced from mannosaccharodilactone by means of alkaline reagents is structurally related to 2 : 5-dimethyl Δ^4-mannosaccharo-3 : 6-lactone 1-methyl ester (VI).

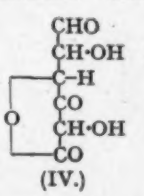
One of the characteristic properties of γ- and δ-lactones prepared from sugars by the oxidation of the reducing group is that they exhibit no reducing activity. Indeed, the conversion of a reducing sugar into a lactone is verified by the fact that the solution no longer reduces Fehling's solution. It is therefore not surprising that Kiliani's discovery (*Ber.*, 1887, **20**, 2710) of a sugar acid dilactone, *l*-mannosaccharodilactone, showing reducing properties excited considerable interest. This dilactone reduces Fehling's solution and reacts in alkaline solution with four atomic proportions of iodine. It also reacts with two atomic proportions of sodium hydroxide under conditions suggestive of a dilactone structure, and two alternative formulæ, (I) and (II), were put forward (Kiliani, *loc. cit.*). Formula (I), containing two four-atom lactone rings, is not considered to be a likely possibility owing to the strain inherent in such a structure, but (II), having two five-atom or γ-lactone rings, is much more probable. Formula (III), not mentioned by Kiliani, must also be taken into account; it contains one five-atom and one six-atom lactone ring. The weakly acid character of a freshly prepared aqueous solution of the dilactone and its relatively slow rate of mutarotation favour the structure (II) rather than (III), since the latter, having a δ-lactone ring, would be expected to show relatively rapid mutarotation. It should be borne in mind, however, that, although the rate of mutarotation displayed by a monolactone can be employed in aiding structural studies (Haworth, " The Constitution of the Sugars," 1929), yet it does not follow that the same general rule, relating mutarotation and ring structure, will apply to dilactones. Since this work was

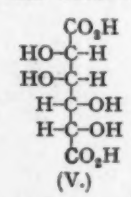

(I.)


(II.)


(II*a*.)


(III.)


(IV.)

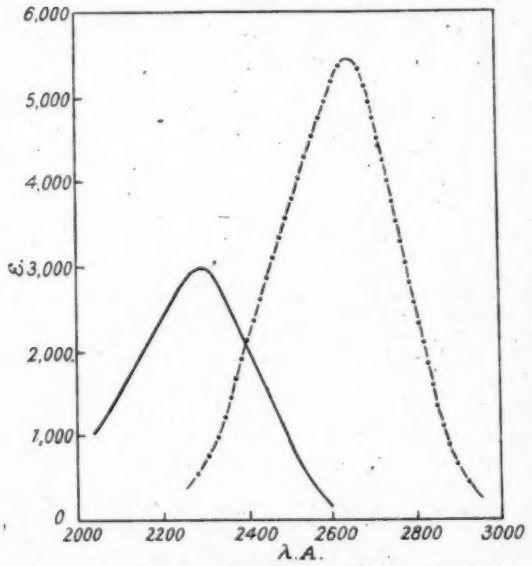

(V.)

completed, it has been shown that mannosaccharodilactone has the structure (II), because upon treatment with moist diazomethane it affords a dimethyl derivative (II*a*), the structure of which follows from the observ-

ation that the diamide derived from it gives no sodium *iso*cyanate when treated with sodium hypochlorite (Schmidt and Kraft, *Ber.*, 1941, **74**, 33). It was recognised by Kiliani that none of these structures explained the unexpected reducing properties of the dilactone, and he suggested that some molecular rearrangement had taken place with the formation of a substance possessing an aldehyde and a keto-group as in (IV). Nevertheless, the ease of formation of a mono- and a di-phenylhydrazide and a diamide from the compound were in accordance with the simple dilactone structure.

The view that the reducing properties were an inherent characteristic of the dilactone itself and not due to some reducing impurity (see Fischer, *Ber.*, 1891, **24**, 539) was substantiated by the production of two series of salts from the dilactone, in one set of which the reducing capacity was retained whereas the other series behaved like the salts of a normal sugar acid in that they showed no reducing activity. Thus, for example, it was demonstrated that when an aqueous solution of the dilactone was allowed to react with an excess of potassium hydroxide, an intense yellow colour developed and the potassium salt isolated from the reaction mixture still reduced Fehling's solution. When, however, the diamide from the dilactone was heated with potassium hydroxide, a different potassium salt was formed which displayed no reducing activity. Moreover, since this non-reducing potassium salt, obtainable also by titration of crystalline mannosaccharic acid (V) with potassium hydroxide (Rehorst, *Ber.*, 1932, **65**, 1475), could not be transformed into the reducing salt except through intermediate conversion into the dilactone, it was evident that the formation in solution of the reducing compound is due to isomerisation taking place in the dilactone itself. This view is supported by the fact that aqueous solutions of the dilactone undergoing mutarotation to the open-chain acid (V) show a gradual decrease in reducing activity, and conversely aqueous solutions of mannosaccharic acid undergo lactonisation upon heating with a consequent increase in reducing power (Rehorst, *loc. cit.*).

FIG. 1.



Mannosaccharodilactone in aqueous alkaline solution (*c*, 3 *mg.*/100 *c.c.*).
Mannosaccharodilactone after treatment with aqueous alkali followed by acid (*c*, 15 *mg.*/100 *c.c.*).

We have obtained some insight into this problem of the reducing activity of mannosaccharodilactone in alkaline solution by the application of spectrophotometry. A freshly prepared aqueous solution of this dilactone shows no selective absorption band in the ultra-violet region of the spectrum, but immediately upon the addition of an excess of sodium hydroxide an intense characteristic band appears at λ 2630 A., accompanied by the development of reducing activity; for instance, such an alkaline solution reduces Fehling's solution, reacts with alkaline iodine to give iodoform, and immediately decolorises potassium permanganate. Furthermore, on acidification of this alkaline solution, the absorption band moves to λ 2290 A. (Fig. 1) and it is noteworthy that the acidified solution still shows reducing activity towards Fehling's solution and neutral potassium permanganate solution. It is evident from these observations that acidification of an alkaline solution of the dilactone gives rise to an active substance in solution, a deduction previously made by Rehorst from his investigations of the reducing activity shown by the sodium and the potassium salt derived from the dilactone. In addition to showing the reducing properties of alkaline solutions of the dilactone, the acidified solution exhibiting a band at λ 2290 A. also behaves as if an unsaturated substance is present, since it reacts quickly with chlorine and with bromine. Treatment of the acidified solution with chlorine causes a profound change in the structure of the reactive isomeric substance, since the acid solution no longer displays selective absorption; this observation suggests, but does not prove, that the substance responsible for the absorption band at λ 2290 A. in acidified solutions is also unsaturated in character and reacts with chlorine. The reaction with chlorine, which will be referred to in a later communication, appears to be complex in nature, for, although the acidified solution after treatment with chlorine is quite transparent to ultra-violet light, it still shows reducing action towards Fehling's solution.

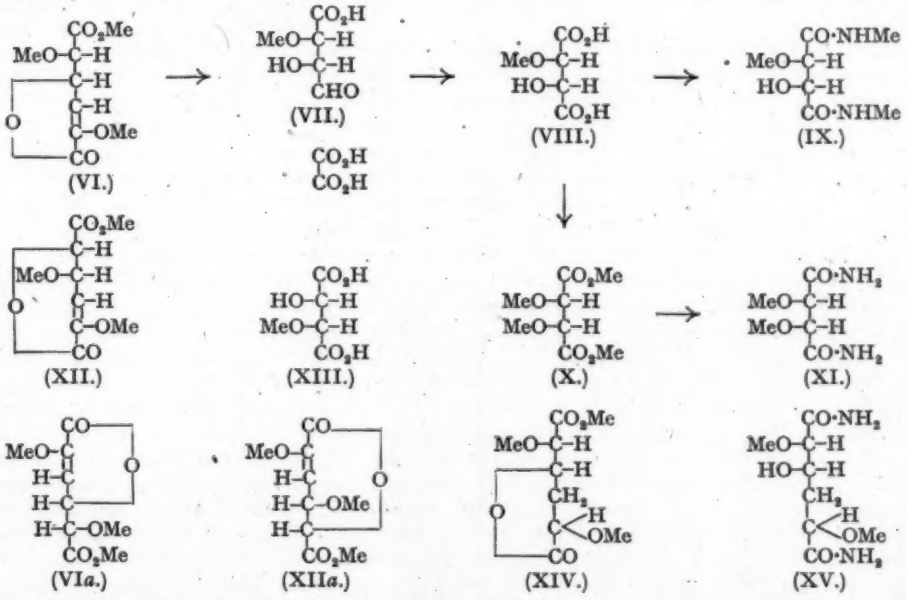
A comparison of these peculiar properties of the active isomeric form of mannosaccharodilactone with those of *l*-ascorbic acid (Herbert, Hirst, Percival, Reynolds, and Smith, J., 1933, 1270) shows that they bear a striking resemblance to each other. For example, ascorbic acid reduces Fehling's solution, decolorises neutral potassium permanganate, and reacts with chlorine and bromine; it also displays a band at λ 2450 A. in acidified aqueous solution, moving to λ 2650 A. on addition of sodium hydroxide; the band at λ 2450 A. disappears upon the addition of a slight excess of chlorine. It will be observed that these properties are very similar to those shown by the active form of mannosaccharodilactone, mentioned previously. Solutions of the active form of this dilactone and of ascorbic acid do, however, differ in some important respects; for instance, the active form of the dilactone requires heating with Fehling's solution before reduction becomes manifest, whereas ascorbic acid reduces Fehling's solution in the cold. Further, ascorbic acid reacts immediately with two atomic pro-

portions of iodine in acid solution, whereas solutions of the active form of mannosaccharodilactone react with only a small amount of iodine. This difference is apparently to be traced to the fact that the reaction of ascorbic acid with chlorine, bromine, and iodine is one of oxidation, whereas that occurring between the active form of mannosaccharodilactone and chlorine or bromine is to some extent one of addition.

Despite these dissimilarities, the question arises as to whether the substance derived from the dilactone by means of alkaline reagents, which exhibits the peculiar reducing properties, possesses, like ascorbic acid, a five-atom lactone ring having an unsaturated conjugated system of double bonds.

The problem was simplified by spectrophotometric measurements which indicated that the peculiar isomerisation of mannosaccharodilactone brought about by the agency of alkaline reagents can also be effected by treatment of the dilactone with diazomethane and with Purdie's reagents (Haworth, *J. Soc. Chem. Ind.*, 1933, **52**, 482). Since these two reagents effect not only isomerisation but methylation at the same time, it was decided to determine the structure of the now stabilised isomeric methyl derivative and then proceed to the elucidation of the structure of the more reactive unmethylated active form of mannosaccharodilactone itself.

One treatment of mannosaccharodilactone with diazomethane gave a partly methylated product, but repeated treatment afforded a fully methylated product, and this upon fractional distillation yielded an unsaturated methyl ester (VI). This ester was also produced by the methylation with silver oxide and methyl iodide of the partly methylated material obtained from the dilactone by one treatment with diazomethane, and it resulted also from the direct methylation of mannosaccharodilactone with Purdie's reagents (cf. Schmidt and Kraft, *loc. cit.*).



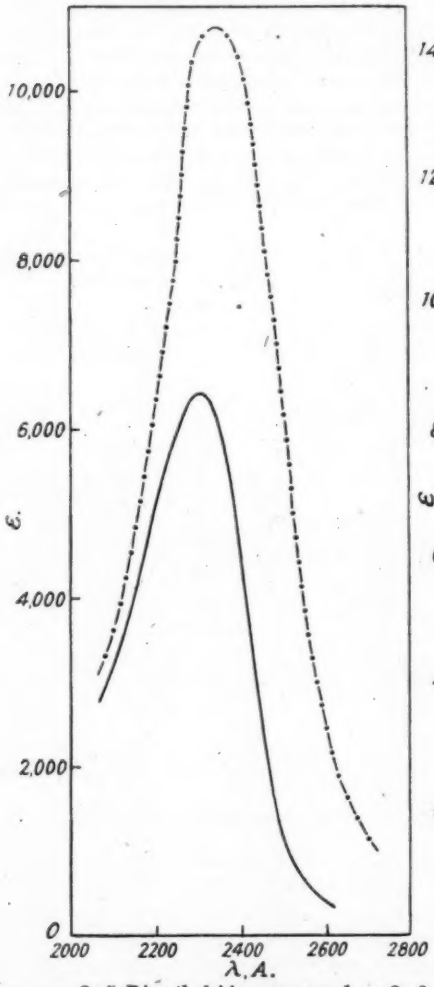
The ester (VI) was non-reducing (to Fehling's solution) but its unsaturated character was shown by its reactivity towards chlorine and bromine. Moreover, an aqueous solution of the ester (VI) exhibited strong selective absorption at λ 2290 A. (see Fig. 2), the band disappearing upon addition of alkali. On acidification of the alkaline solution, this band reappeared, though its intensity was considerably diminished. On keeping the acid solution, however, the intensity gradually increased until it eventually reached that originally shown by the ester. A comparison of the band shown by (VI) with that displayed by 2 : 3 : 5 : 6-tetramethyl *l*-ascorbic acid (Fig. 2) (Haworth, Hirst, and Smith, J., 1934, 1556) suggested that the conjugated system present in the latter was closely related to that in (VI). If this was the case, then the disappearance in alkaline solution of the band at λ 2290 A. is probably due to the opening of the conjugated ring system as in the case of 2 : 3 : 5 : 6-tetramethyl *l*-ascorbic acid, which shows a band at λ 2350 A., disappearing upon addition of alkali. On acidification of the alkaline solution of (VI), an open-chain acid would first be formed, and this would slowly undergo lactonisation with the simultaneous production in solution of the original substance showing selective absorption; the gradual increase in the intensity of the band at λ 2290 A. would therefore be expected.

The ester (VI) was proved to be 2 : 5-*dimethyl* Δ^4-*mannosaccharo*-3 : 6-*lactone* 1-*methyl ester* as a result of the following experimental facts. The substance (VI) had the formula $C_9H_{12}O_6$ and contained three methoxyl groups. Aqueous solutions of (VI) were neutral to litmus and Congo-red paper, but on warming, the substance (VI) reacted with 2 equivs. of sodium hydroxide with the elimination of 1 mol. of methyl alcohol, an observation which showed that two potentially acidic groups were present in (VI) and that one of these (that affording the methyl alcohol upon alkaline hydrolysis) was probably a carbomethoxy-group. The unsaturated nature of the compound was suggested by the fact that it reacted with 2 atomic proportions of chlorine, a process attended by the disappearance of the selective absorption band. The substance (VI) also combined with bromine and

immediately decolorised potassium permanganate in alkaline solution; furthermore, it reacted with 3 atomic proportions of iodine in alkaline solution, with the formation of a small amount of iodoform.

When a solution of the unsaturated ester (VI) in glacial acetic acid was subjected to ozonisation, a considerable change in rotation took place, accompanied by the formation of oxalic acid and the aldehydic acid, monomethyl erythuronic acid (VII). The presence of an aldehydic group in the product after removal of the oxalic acid was shown by its strong reducing action towards Fehling's solution. Since fission of the double bond was accompanied by the formation of an additional carboxyl group and an aldehydic group, as well as the loss of a methoxy-group, it follows that the system $-C(OMe)=CH-$ must have been present in the

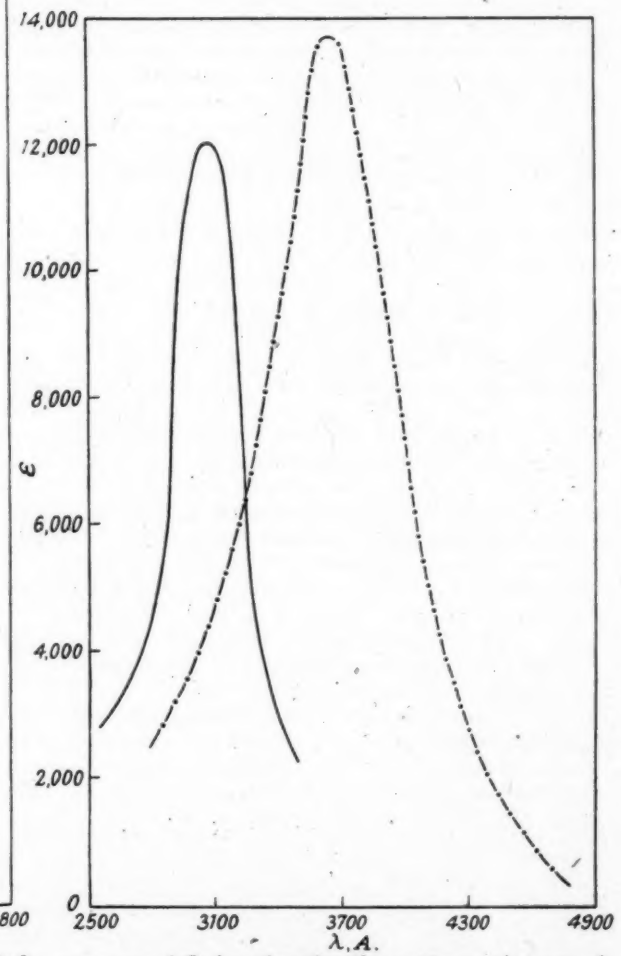
FIG. 2.



——— 2 : 5-*Dimethyl* Δ^4-*mannosaccharo*-3 : 6-*lactone methyl ester in water* (*c*, 5·5 *mg.*/100 *c.c.*).

———•———•——— 2 : 3 : 5 : 6-*Tetramethyl* l-*ascorbic acid in water* (*c*, 4 *mg.*/100 *c.c.*).

FIG. 3.



——— 6-*Carbomethoxy*-3-*methoxy*-α-*pyrone in water* (*c*, 2 *mg.*/100 *c.c.*).

———•———•——— 6-*Carbomethoxy*-3-*methoxy*-α-*pyrone in aqueous alkali* (*c*, *mg.*/100 *c.c.*).

unsaturated ester (VI). The production of a two-carbon fragment (oxalic acid) and a four-carbon fragment (VII) indicates that the double bond must be located between C_4 and C_5, and furthermore, since the two-carbon fragment proved to be oxalic acid and not glyoxylic acid, it is evident that the methoxyl residue eliminated as a result of ozonolysis was attached to C_5 and not to C_4; it also follows from these results that the hydrogen atom present in the aldehydic group of the four-carbon fragment was attached to C_4. Oxidation of the aldehydic acid (VII) with bromine gave a *hydroxymethoxysuccinic acid* (VIII), which was characterised, after conversion into the methyl ester, by its transformation into a crystalline *diamide*. The diamide showed no appreciable rotation in aqueous solution, but the molecular dissymmetry of the acid (VIII) was demonstrated by the fact that its methyl ester yielded a crystalline *bismethylamide* (IX) which had a positive rotation. The acid (VIII) was shown to be stereochemically related to 2 : 3-dihydroxyerythrosuccinic acid (*meso*tartaric acid) because methylation of the methyl ester of (VIII) with Purdie's reagents gave methyl dimethoxyerythrosuccinate

(methyl dimethyl *meso*tartrate) (X), identified by its conversion into the known crystalline dimethoxyerythrosuccinamide (inactive dimethoxysuccinamide) (XI) (Haworth and Hirst, J., 1926, 1858). Although these results established the position of the double bond and proved that the acid (VIII) was a monomethyl derivative of dihydroxyerythrosuccinic acid, it was not clear whether this hitherto unknown hydroxymethoxysuccinic acid had the configuration represented by (VIII), *i.e.*, 3-hydroxy-2-methoxy-*l*-erythrosuccinic acid, or by the only other possible formulation (XIII), *viz.*, 2-hydroxy-3-methoxy-*l*-erythrosuccinic acid which is the enantiomorph of (VIII); both (VIII) and (XIII) upon methylation would yield the same inactive methyl 2 : 3-dimethoxyerythrosuccinate (X). Hence, in addition to the structure (VI) suggested for the original unsaturated methyl ester, the alternative formulation (XII), in which a δ-lactone ring is present, had to be taken into consideration, because ozonisation of a substance having this formulation (XII), followed by bromine oxidation, would give 2-hydroxy-3-methoxy-*l*-erythrosuccinic acid (XIII), one of the two possible structures for the hydroxymethoxyerythrosuccinic acid. It might be suggested that the formation of oxalic acid and the aldehydic acid (VII) could also be explained by the two formulæ (VI*a*) and (XII*a*), in both of which the double bond is situated between C_2 and C_3, but examination of these two formulations shows that they are identical respectively with the two structures (VI) and (XII) now under consideration. Thus it can be seen that if the hydroxymethoxysuccinic acid derived from the aldehydic acid by bromine oxidation is 3-hydroxy-2-methoxy-*l*-erythrosuccinic acid, as in (VIII), then the original unsaturated ester must have the formulation (VI), whereas if the hydroxymethoxysuccinic acid is 2-hydroxy-3-methoxy-*l*-erythrosuccinic acid (XIII), then (XII) is the correct structure of the original unsaturated ester. It is clear therefore that the absolute configuration of the hydroxymethoxysuccinic acid obtained from the aldehydic acid must be ascertained in order to decide which of the two structures (VI) or (XII) is correct.

We have now solved this problem by the synthesis of both the *d*- and the *l*-form of hydroxymethoxyerythrosuccinic acid. One of these, 3-hydroxy-2-methoxy-*l*-erythrosuccinic acid, which is the same as 2-hydroxy-3-methoxy-*d*-erythrosuccinic acid, was prepared from *d*-araboascorbic acid by a series of reactions which leaves no doubt about its structure; the enantiomorph, 2-hydroxy-3-methoxy-*l*-erythrosuccinic acid (3-hydroxy-2-methoxy-*d*-erythrosuccinic acid), was obtained by resolution of the racemic mixture of the hydroxymethoxyerythrosuccinic acids prepared from inactive dihydroxyerythrosuccinic acid (*meso*tartaric acid) by methylation with methyl sulphate and sodium hydroxide (see succeeding paper).

The 3-hydroxy-2-methoxy-*l*-erythrosuccinic acid, obtained from *d*-araboascorbic acid, gave immediately upon treatment with diazomethane the corresponding methyl ester, which yielded a crystalline bismethylamide identical with that (IX) prepared from the acid (VIII). The latter must therefore be 3-hydroxy-2-methoxy-*l*-erythrosuccinic acid, and hence the original unsaturated substance must have the structure shown in (VI) and not that shown in (XII). The 3-hydroxy-2-methoxy-*d*-erythrosuccinic acid prepared from *meso*tartaric acid afforded a bismethylamide which proved to be the enantiomorph of (IX).

Further evidence in support of the structure (VI) assigned to the unsaturated methyl ester was provided by an examination of the deoxy-compound (XIV) obtained from (VI) by hydrogenation. Reduction of (VI) was effected by means of sodium amalgam, and there was obtained a deoxy-acid which upon treatment with boiling methyl-alcoholic hydrogen chloride gave rise to the *deoxy-lactone methyl ester* (XIV). The fact that aqueous and alkaline solutions of this ester showed no selective absorption indicated that saturation of the double bond between C_4 and C_5 had been effected. Saturation of the double bond was also carried out by catalytic hydrogenation. Treatment of the 4-deoxy-ester (XIV) with ammonia in methyl alcohol furnished a crystalline *diamide* (XV) which showed a negative Weerman test for α-hydroxy-amides (*Rec. Trav. chim.*, 1917, **36**, 16). Methoxy-groups must therefore be located in positions 2 and 5 of (XV) and therefore of (XIV), and hence it follows that the hydroxy-group set free by opening of the lactone ring during the conversion of the deoxy-lactone methyl ester (XIV) into the diamide (XV) must occupy position 3. The lactone ring in the deoxy-compound (XIV), and therefore in the 2 : 5-dimethyl Δ^4-mannosaccharolactone methyl ester, must engage carbon atoms C_3 and C_6.

It is noteworthy that saturation of the double bond between C_4 and C_5 in (VI) introduces further dissymmetry into the molecule at C_5, and consequently two 4-deoxy-lactone methyl esters are theoretically possible. Only one crystalline diamide was isolated, however, and in this the spatial arrangement of the hydrogen and the methoxy-group at C_5 still remains to be determined.

From the foregoing it will be seen that there is a close relationship betwen the properties of a solution of 2 : 5-dimethyl Δ^4-mannosaccharo-3 : 6-lactone 1-methyl ester (VI) and a solution of the " active " form of mannosaccharodilactone prepared from the latter by the agency of alkali, followed by acid; *e.g.*, both solutions show intense bands at λ 2290 A., and both react with chlorine, bromine, alkaline permanganate, and alkaline iodine. In some respects, however, they differ; for example, a solution of the " active " form of the dilactone reduces Fehling's solution, whereas (VI) does not; but such differences as do exist are probably attributable to the fact that (VI) is a methylated compound. Such considerations led us to the view that the substance responsible for the remarkable and unexpected behaviour of mannosaccharodilactone (after treatment with alkaline reagents) has a structure analogous to (VI).

Experimental.

Mannosaccharodilactone.—(a) *From mannose.* A solution of mannose (10 g.) in nitric acid (25 c.c., *d* 1·2) was heated on the water-bath for 48 hours at 55°. The yellow solution was then concentrated slowly in an open dish at 80°. Before darkening of the product occurred and whilst it still contained some solvent (water and nitric acid), the concentration

was completed in a vacuum over potassium hydroxide. The almost colourless crystalline solid was triturated with ethyl alcohol–ether and filtered off. Purification of the compound by recrystallisation from ethyl alcohol gave mannosaccharodilactone as silky plates, m. p. 189°, $[\alpha]_{5780}^{18°}$ +214° (initial value in water; *c*, 1·1) (yield 2·5 g.).

The following modification gave a slightly better yield. The same quantities of mannose and nitric acid were heated on the water-bath for 4 hours at 60°. The temperature was then raised to 85°, and the oxidation continued for a further ½ hour. Removal of the nitric acid by distillation under diminished pressure gave an almost colourless syrup which crystallised spontaneously. The crystals of mannosaccharodilactone were separated by trituration with ethyl alcohol containing a little ether, and recrystallised from ethyl alcohol; m. p. 187° (yield, 3·0 g.).

(b) *From α-methylmannopyranoside.* Oxidation of α-methylmannoside with nitric acid by the above modified method gave a 30% yield of mannosaccharodilactone.

Aqueous solutions of mannosaccharodilactone show no selective absorption band (tested at 200 mg.%) and are unaffected by chlorine, bromine, iodine, and potassium permanganate. Upon addition of excess of N-sodium hydroxide to a solution of the dilactone, a band appears at λ 2630 A. (ε, 5,500; *c*, 3·0 mg.%); acidification of this alkaline solution causes this band to move to λ 2290 A. (ε, 3,000; *c*, 15 mg.%) (see Fig. 1). This acidified solution contains what may be referred to as the active form of mannosaccharodilactone; it reacts immediately with chlorine, bromine, and potassium permanganate. Alkaline solutions of mannosaccharodilactone readily reduce hot Fehling's solution and immediately decolorise potassium permanganate in the cold.

The Action of Alkaline Iodine upon Mannosaccharodilactone.—In accordance with the findings of Kiliani and Rehorst (*locc. cit.*) mannosaccharodilactone was found to react in alkaline solution with approximately 4 atomic proportions of iodine.

A solution of mannosaccharodilactone (0·303 g.) in 0·1N-sodium hydroxide (87 c.c.) was treated with 0·1N-iodine (50 c.c.) at room temperature. After 90 mins., the solution was acidified by addition of N-sulphuric acid (10 c.c.), and the excess of the iodine was titrated with 0·1N-sodium thiosulphate (27·7 c.c.). A blank experiment was carried out at the same time. Hence in alkaline solution 1 mol. of mannosaccharodilactone reacts with 3770 c.c. of N-iodine or approximately 4 atomic proportions. A small amount of iodoform is produced in this alkaline iodine oxidation of mannosaccharodilactone.

Oxalic acid is also formed when alkaline iodine is allowed to react with mannosaccharodilactone. The dilactone (0·1 g.) in water (5 c.c.) was treated with N-sodium hydroxide (5·7 c.c.) and iodine (4·0 c.c. containing 0·4 g. of iodine). After the mixture had stood for 15 mins. at room temperature, it was acidified with glacial acetic acid (2 c.c.) and filtered to remove iodoform. A stream of sulphur dioxide was passed through the solution to decolorise the iodine, and then phenylhydrazine (0·15 g.) was added. Phenylhydrazine oxalate readily separated as large plates, m. p. and mixed m. p. 178°. The complex nature of this alkaline iodine oxidation of mannosaccharodilactone is indicated by the fact that occasionally a yellow phenylhydrazine derivative, m. p. 212°, was obtained (see Rehorst, *Ber.*, 1938, **71**, 923).

The Effect of Chlorine upon the "Active" Form of Mannosaccharodilactone.—A solution of mannosaccharodilactone (0·1 g.) in water (5 c.c.) was treated with 0·1N-sodium hydroxide (13 c.c.) for 1 min. at room temperature. The solution was acidified by addition of 0·1N-sulphuric acid (13·5 c.c.) and, now containing the "active" form of mannosaccharodilactone showing a band at λ 2290 A., it was titrated with 0·085N-chlorine water (13·3 c.c.). The end-point was determined by starch–iodide paper. It may be calculated that 1 mol. of mannosaccharodilactone after the above treatment with alkali followed by acid reacts with 19,700 c.c. of 0·1N-chlorine water, or approximately 2 atomic proportions.

When freshly prepared, the chlorine oxidation solution referred to above shows no selective absorption band and has $[\alpha]_{5780}^{18°}$ +84° (initial value); +78° (after 2½ hours); +65° (8 hours); +45° (22 hours); +35° (30 hours); +25° (40 hours); +15° (60 hours); +12·5° (80 hours); +17° (170 hours); +19° (200 hours); +24° (380 hours, constant value).

The Effect of Potassium Permanganate upon the "Active" Form of Mannosaccharodilactone.—A solution of the dilactone (0·1 g.) in water (10 c.c.) was treated with N-sodium hydroxide (3 c.c.), followed 3 mins. later by N-sulphuric acid (5 c.c.). After the addition of a slight excess of 0·1N-potassium permanganate (6·6 c.c.) (Calc. for 1 atomic proportion of oxygen, 11·6 c.c.) the oxidised solution showed $[\alpha]_{5780}^{18°}$ +87° (initial value).

Both methods of oxidation appear to have the same effect upon solutions containing the "active" form produced from mannosaccharodilactone by the agency of alkaline reagents, and from the nature of the mutarotation of a freshly prepared oxidised solution the active principle would appear to be of the nature of a lactone.

Methylation of Mannosaccharodilactone.—(A) *With silver oxide and methyl iodide.* Finely ground mannosaccharodilactone (4 g.) was boiled with methyl iodide in the presence of silver oxide in the usual manner, the process of methylation being facilitated by addition of just sufficient methyl alcohol to dissolve the dilactone. After the first methylation in this way, the product, isolated by means of methyl alcohol, was soluble in methyl iodide. After three more methylations with Purdie's reagents (no methyl alcohol added) the syrupy product, the methoxyl content of which had reached a maximum, was distilled, giving: Fraction I (0·48 g.), b. p. (bath temp.) 130°/0·01 mm., $n_D^{18°}$ 1·4400, $[\alpha]_D^{18°}$ −9·5° in water (*c*, 0·8); equiv., 123 (Found: OMe, 56·0%). This fraction was mainly methyl dimethyl *meso*tartrate, for upon treatment with methyl-alcoholic ammonia it gave dimethyl *meso*tartramide, m. p. and mixed m. p. 258° (after recrystallisation from hot water) (Found: OMe, 35·2. Calc. for $C_6H_{12}O_4N_2$: OMe, 35·2%). Fraction II (1·3 g.), b. p. (bath temp.) 140°/0·10 mm., $n_D^{19°}$ 1·4645, $[\alpha]_D^{18°}$ −16·5° in water (*c*, 0·7); equiv., 118 (Found: OMe, 47·5, and after saponification by heating with barium hydroxide, OMe, 30·8%). Fraction III (0·6 g.), b. p. (bath temp.) 140—142°/0·01 mm., $n_D^{19°}$ 1·4675, $[\alpha]_D^{18°}$ −15° in water (*c*, 1·0); equiv., 113 (Found: OMe, 46·7, and after saponification with barium hydroxide, OMe, 29·6%). When an aqueous solution of this fraction was treated with a slight excess of sodium hydroxide in the cold, the rotation changed immediately from $[\alpha]_D^{18°}$ −15° to −26°. Fraction IV (1·82 g.), b. p. (bath temp.) 142—145°/0·01 mm., $n_D^{19°}$ 1·4670, $[\alpha]_D^{18°}$ −7° in water (*c*, 0·7); equiv., 108 [Found: OMe, 45·6, and after saponification by heating with barium hydroxide, OMe, 31·0%. $C_9H_{18}O_6$ (2 : 5-*dimethyl* Δ⁴-*mannosaccharolactone methyl ester*) requires OMe, 43·1%; equiv., 108, and after the loss of one ester OMe group by saponification the residual methoxyl value should be 28·7%]. The high methoxyl values of fractions II, III, and IV are due to the presence of some 2 : 3 : 5 (or 2 : 4 : 5)-trimethyl mannosaccharolactone methyl ester, because treatment of small portions of each fraction with methyl-alcoholic ammonia afforded (in small yield) the crystalline *diamide* of 2 : 3 : 5 (or 2 : 4 : 5)-trimethyl mannosaccharic acid, m. p. 258°, $[\alpha]_D^{18°}$ −40° in water (*c*, 1·1). This diamide showed a negative Weerman test for α-hydroxy-amides when the test was performed under the conditions used previously (Smith, J., 1939, 753) (Found: C, 43·2; H, 7·0; N, 11·4; OMe, 37·9. $C_9H_{18}O_6N_2$ requires C, 43·2; H, 7·2; N, 11·2; OMe, 37·2%).

Each of the fractions II, III, and IV, consisting mainly of 2 : 5-dimethyl Δ⁴-mannosaccharo-3 : 6-lactone 1-methyl ester, showed in aqueous solution an intense selective absorption band at λ 2290 A. (ε, 5,000; *c*, 5 mg.%) (see Fig. 2), disappearing on addition of sodium hydroxide, reappearing slowly on acidification.

(B) *With diazomethane.* A solution of mannosaccharodilactone (7 g.) in dry methyl alcohol was cooled in a freezing mixture and treated with excess of an ice-cold ethereal solution of diazomethane for 2 days at −5°. Removal of the solvent under diminished pressure gave a thick syrup, which was non-reducing to Fehling's solution and showed $[\alpha]_D^{18°}$ −24° in 50% aqueous methyl alcohol (*c*, 1·3) (Found: OMe, 36·6%). The syrupy product showed an intense absorption band at λ 2300 A.; ε, 5,000 (*c*, 5 mg.% in water).

After three treatments with ethereal diazomethane, the product had a methoxyl value of 40·5% but it was still insoluble in ether. It was therefore dissolved in a small volume of methyl alcohol and re-treated with ethereal diazomethane for 3 days at −5°. After this fourth treatment the product had $n_D^{20°}$ 1·4795 (Found : OMe, 41·1%). The reaction with ethereal diazomethane was carried out a fifth time for 4 days at −5°. The product was isolated by removal of the solvent and distilled, giving Fraction I (0·3 g.), b. p. (bath temp.) 180°/0·02 mm., $n_D^{18°}$ 1·4934; OMe, 44·3%. The distillate crystallised spontaneously, and after trituration with aqueous ethyl alcohol to remove adhering syrup, followed by recrystallisation from ethyl alcohol, the crystals had m. p. 140° (Found: C, 52·1; H, 6·5; OMe, 49·8. Calc. for $C_{10}H_{14}O_6$: C, 52·2; H, 6·1; OMe, 53·9%). An ethyl-alcoholic solution of the crystals showed no appreciable rotation; they were insoluble in water, and decolorised bromine water. In 50% aqueous ethyl alcohol the crystalline substance showed an intense band at λ 3180 A. (ε, 17,000; *c*, 2 mg.%); no change in the position or intensity of the band occurred on making the solution alkaline. The material was unaffected by treatment with methyl-alcoholic ammonia.

Fraction II (2·5 g.) had b. p. (bath temp.) 180°/0·02 mm., $n_D^{18°}$ 1·4870, $[\alpha]_D^{18°}$ −20° in water (*c*, 1·0); OMe, 43·9, and after saponification with barium hydroxide, OMe, 25·0%, corresponding (by diff.) to an ester methoxyl content of 18·9%.

Fraction III (3·5 g.) had b. p. (bath temp.) 180°/0·02 mm., $n_D^{18°}$ 1·4820, $[\alpha]_D^{18°}$ −18° in water (*c*, 1·2) (Found : OMe, 42·3; and, after saponification, OMe, 24·2%, corresponding to an ester methoxyl content of 18·1%).

Both fractions II and III, consisting mainly of 2 : 5-dimethyl Δ^4-mannosaccharolactone methyl ester, had an intense band at λ 2290 A. (ε, 6,500; *c*, 5 mg.%). The presence of a small amount of 6-carbomethoxy-3-methoxy-α-pyrone (see below) can sometimes be detected in such fractions of 2 : 5-dimethyl Δ^4-mannosaccharo-3 : 6-lactone 1-methyl ester by an absorption band at *ca.* λ 3050 A. (ε, 2,000; *c*, 5 mg.%). Aqueous solutions of both fractions reacted with chlorine, decolorised bromine and alkaline potassium permanganate, and reacted with iodine (3 atoms approx.) in alkaline solution.

Further distillation of the residue in the flask after removal of fractions I, II, and III gave a crystalline distillate (0·1 g.), b. p. 180°/0·01 mm., m. p. 212° (after recrystallisation from aqueous ethyl alcohol). This substance was sparingly soluble in cold water and cold alcohol, soluble in acetone, and insoluble in ether and light petroleum. It proved to be identical with 6-carbomethoxy-3-methoxy-α-pyrone, with which it gave no depression of the m. p. (Haworth, Hirst, and Jones, J., 1938, 710; Schmidt, Dippold, and Zeisser, *Ber.*, 1937, **70**, 2402) (Found : C, 52·4, H, 4·1; OMe, 31·5. Calc. for $C_8H_8O_5$: C, 52·2; H, 4·4; OMe, 33·7%). Aqueous solutions of this α-pyrone exhibit a strong band at λ 3070 A. (ε, 12,000; *c*, 2 mg.%), moving to λ 3680 A. (ε, 14,000; *c*, 2 mg.%) upon addition of sodium hydroxide (see Fig. 3).

(C) *With diazomethane followed by Purdie's reagents.* A solution of mannosaccharodilactone (5 g.) in methyl alcohol was treated for 2 days at −5° with an excess of an ethereal solution of diazomethane. The persistent yellow colour of the solution after this time indicated that excess of diazomethane was present. The solution was filtered to remove a small amount of flocculent material, and then evaporated under diminished pressure to give a syrup (5·6 g.) (Found : OMe, 37·0%). The pale yellow syrup had a band at λ 2300 A. (ε, *ca.* 4000; *c*, 5 mg.%).

A solution of this syrup (5·6 g.) in methyl iodide (10 c.c.) was boiled under reflux in the presence of silver oxide, fresh additions of which were made every hour; the reaction mixture was frequently shaken to promote methylation. After removal of the solvent the residue was extracted several times with acetone, filtered, and concentrated to a syrup (Found : OMe, 40·2, after two Purdie treatments; 43·0, after 4. Calc. for $C_9H_{12}O_6$: OMe, 43·1%). The syrupy product was purified by extraction with ether and then distilled, giving Fraction I (0·3 g.), b. p. (bath temp.) 145°/0·04 mm., $n_D^{21°}$ 1·4736; OMe, 44·2%. This pale yellow liquid crystallised spontaneously; after trituration with ethyl alcohol to remove adhering syrup, the crystals had m. p. 140°, and were identical with those obtained by the methylation as in (A), above. Fraction II (3·6 g.), b. p. (bath temp.) 152—158°/0·04 mm., $n_D^{21°}$ 1·4688; OMe, 43·4%; $[\alpha]_D^{18°}$ −25° in water (*c*, 1·0), consisting mainly of 2 : 5-dimethyl Δ^4-mannosaccharolactone methyl ester, showed in aqueous solution an intense band at λ 2290 A. (ε, 4000; *c*, 5 mg.%).

Ozonisation of 2 : 5-*Dimethyl* Δ^4-*Mannosaccharo*-3 : 6-*lactone* 1-*Methyl Ester.*—Fractions II, III, and IV of this ester prepared by method (A) were combined, dissolved in glacial acetic acid (20 c.c.), and the solution subjected to the action of a stream of ozonised oxygen at room temperature. Initially, the solution had $[\alpha]_D$ −31° and after 10 hours' ozonolysis it showed $[\alpha]_D$ +8° (constant value). The solution at this stage did not reduce Fehling's solution. Removal of the acetic acid by distillation under diminished pressure gave a syrup, insoluble in water, which was probably the ozonide. Simultaneous addition and distillation of water under reduced pressure decomposed this ozonide, and the syrupy product became readily soluble in water, strongly reducing towards Fehling's solution on gentle warming, and it reacted strongly acid to Congo-red paper; the aqueous distillate contained no glyoxylic acid (tested with albumin and sulphuric acid). Oxalic acid was present in the syrupy product, for when an aqueous solution of a small portion of it was made just alkaline with sodium hydroxide and then acidified with acetic acid it gave a white precipitate of calcium oxalate upon addition of calcium chloride. The syrupy product showed a negative test for glyoxylic acid.

A solution of the syrup (obtained from the ozonolysis as described above) in water (15 c.c.) was neutralised with barium carbonate and filtered to remove the barium oxalate and the excess of the barium carbonate. The filtrate (still strongly reducing to Fehling's solution on slight warming) was then treated with bromine (2 c.c.) at room temperature for 2 days, the solution then no longer reducing Fehling's solution. The excess of the bromine was removed by aeration; the solution was neutralised with silver oxide, filtered before and after treatment with hydrogen sulphide, and then evaporated to dryness under diminished pressure. The glassy product (an acid barium salt) reacted acid to Congo-red paper and gave a positive test for barium.

This non-reducing acid barium salt of 3-hydroxy-2-methoxy-*l*-erythrosuccinic acid was boiled for 8 hours with 3% methyl-alcoholic hydrogen chloride (100 c.c.). The solution was cooled, neutralised with silver carbonate, filtered, and evaporated to dryness under reduced pressure. Distillation of the syrup thus obtained gave : Fraction I (0·86 g.), b. p. (bath temp.) 105—110°/0·01 mm., $n_D^{17°}$ 1·4440, $[\alpha]_D^{18°}$ −43° in methyl alcohol (*c*, 0·8) [Found : OMe, 51·6%; equiv., 105. $C_7H_{12}O_6$ (*methyl* 3-*hydroxy*-2-*methoxyerythrosuccinate*) requires OMe, 48·5%; equiv., 96]. Fraction II (0·56 g.), b. p. (bath temp.) 125°/0·01 mm., $n_D^{17°}$ 1·4470, $[\alpha]_D^{18°}$ −32·4° in methyl alcohol (*c*, 0·7) (Found : OMe, 54·2%; equiv., 131). Fraction III (0·27 g.), b. p. (bath temp.) 135°/0·01 mm., $n_D^{17°}$ 1·4500, $[\alpha]_D^{18°}$ −21·5° in methyl alcohol (*c*, 0·4) (Found : OMe, 52·0%; equiv., 130. Calc. for $C_{10}H_{16}O_7$ (trimethyl mannosaccharolactone methyl ester) : OMe, 50·0%; equiv., 124].

Examination of Fraction I *and Identification of Methyl* 3-*Hydroxy*-2-*methoxy*-l-*erythrosuccinate.*—(a) *Complete methylation of Fraction* I *with Purdie's reagents.* One methylation of Fraction I (120 mg.) with silver oxide and methyl iodide gave methyl dimethoxyerythrosuccinate (methyl dimethyl *meso*tartrate), $n_D^{18°}$ 1·4350. Treatment of this ester with methyl-alcoholic ammonia for 2 days at −5° readily gave the diamide of dimethoxyerythrosuccinic acid (inactive dimethoxysuccinamide), m. p. and mixed m. p. 258° (decomp.) after recrystallisation from water. This amide was optically inactive (Found : C, 40·7; H, 6·3; OMe, 35·3; N, 15·7. Calc. for $C_8H_{12}O_4N_2$: C, 40·9; H, 6·8; OMe, 35·2; N, 15·9%).

(b) *Isolation of* 3-*hydroxy*-2-*methoxy*-l-*erythrosuccinamide.* A solution of Fraction I (250 mg.) in methyl alcohol (3 c.c.), cooled to 0°, was saturated with dry ammonia. After 2 days' keeping at −5°, some crystals of the diamide of 2 : 3 : 5-trimethyl mannosaccharic acid were separated by decantation (m. p. 257°, decomp., after crystallisation from aqueous alcohol). Evaporation of the methyl-alcoholic ammonia solution, decanted from the crystals of the trimethyl mannosaccharamide, gave a crystalline residue which furnished a further small amount of 2 : 3 : 5-trimethyl mannosaccharamide upon crystallisation from aqueous ethyl alcohol. Evaporation of the aqueous ethyl-alcoholic mother-

liquors gave the *amide* of 3-hydroxy-2-methoxy-*l*-erythrosuccinic acid, m. p. 153° (after recrystallisation from methyl alcohol–ethyl alcohol), showing no appreciable rotation in water (Found: C, 37·1; H, 6·2; OMe, 19·0; N, 17·3. $C_5H_{10}O_4N_2$ requires C, 37·0; H, 6·2; OMe, 19·1; N, 17·3%).

(c) *Isolation of the bismethylamide of 3-hydroxy-2-methoxy-l-erythrosuccinic acid.* Treatment of the remaining portion of Fraction I with methyl-alcoholic methylamine for 2 days at −5°, followed by removal of the solvent, gave a crystalline product in good yield. After recrystallisation from ethyl acetate the *bismethylamide* of 3-hydroxy-2-methoxy-*l*-erythrosuccinic acid had m. p. 136°, $[\alpha]_D^{18°}$ +10·7° in water (*c*, 3·2). This bismethylamide proved to be identical with a synthetic specimen of that of 3-hydroxy-2-methoxy-*l*-erythrosuccinic acid prepared from *d*-araboascorbic acid (see succeeding paper) (Found: C, 44·3; H, 7·35; OMe, 16·2; N, 14·5. $C_7H_{14}O_4N_2$ requires C, 44·2; H, 7·4; OMe, 16·3; N, 14·7%).

Examination of Fractions II *and* III.—Both these fractions upon treatment with methyl-alcoholic ammonia gave in good yield the *diamide* of 2 : 3 : 5- (or 2 : 4 : 5-)trimethyl mannosaccharic acid, m. p. 258° (decomp.), $[\alpha]_D^{20°}$ −41° in water (*c*, 0·6 after recrystallisation from water) (Found: C, 43·2; H, 7·0; OMe, 37·8; N, 11·05. $C_9H_{18}O_6N_2$ requires C, 43·2; H, 7·3; OMe, 37·2; N, 11·2%). This diamide gave a negative Weerman reaction for α-hydroxy-amides.

In a preliminary experiment, the syrupy acid product obtained from the ozonisation of the syrup containing 2 : 5-dimethyl Δ⁴-mannosaccharolactone methyl ester and some 2 : 3 : 5-trimethyl saccharolactone methyl ester was treated directly with methyl-alcoholic ammonia. Since the ozonolysis product contained oxalic acid, the monomethyl ester of hydroxymethoxy-*l*-erythrosuccinic acid, and trimethyl mannosaccharolactone methyl ester, there were obtained ammonium oxalate, 2 : 3 : 5-trimethyl mannosaccharamide, m. p. 254°, and the *half-amide ammonium* salt of 3-hydroxy-2-methoxy-*l*-erythrosuccinic acid, m. p. 181° (decomp.) (after crystallisation from methyl alcohol) (Found: C, 33·5; H, 6·8; OMe, 17·6; N, 14·8. $C_5H_{12}O_5N_2$ requires C, 33·3; H, 6·7; OMe, 17·2; N, 15·6%). The last, however, was of no use for identification purposes, since it proved difficult to purify.

Ozonisation of the 2 : 5-dimethyl Δ⁴-mannosaccharolactone methyl ester prepared by method (B). A solution of Fractions I and II (5·7 g.) in glacial acetic acid (30 c.c.) was subjected to ozonolysis for 17 hours at room temperature; $[\alpha]_D$ −41° (initial value); −32° (after ¼ hour); −1·2° (after 3½ hours); −5° (after 5 hours); −2·5° (after 6½ hours); +2·5° (after 9½ hours; constant value for 7½ hours). The solution was freed from acetic acid by distillation under diminished pressure, and the ozonide was decomposed by simultaneous addition and distillation of water. All traces of acetic acid were removed by evaporation to dryness. The acid product contained oxalic acid and was strongly reducing to Fehling's solution. The residue was dissolved in water (20 c.c.), and the solution neutralised with barium carbonate, and filtered to remove barium oxalate and excess barium carbonate. Evaporation of the filtrate afforded a pale yellow glassy solid which contained barium and reduced Fehling's solution actively on warming. This product was dissolved in water (50 c.c.) and divided into two portions D (20 c.c.) and E (30 c.c.).

Treatment of portion D with bromine. This solution containing the barium salt of the aldehydic acid (2-methyl *l*-erythuronic acid) was oxidised with bromine (1 c.c.) for 2 days at room temperature, the solution then no longer reducing Fehling's solution. The excess of bromine was removed by aeration, the solution neutralised with silver oxide, filtered before and after treatment with hydrogen sulphide, and then evaporated to dryness under reduced pressure. The dry acid barium salt was boiled for 8 hours with 3% methyl-alcoholic hydrogen chloride (100 c.c.). Neutralisation of the mineral acid with silver carbonate, followed by removal of the solvent, gave a fairly mobile liquid which distilled giving: Fraction I, methyl 3-hydroxy-2-methoxy-*l*-erythrosuccinate (0·53 g.), b. p. (bath temp.) 110°/0·02 mm., $n_D^{18°}$ 1·4440, $[\alpha]_D^{18°}$ −31° in water (c, 0·8), $[\alpha]_D^{18°}$ −49·5° in methyl alcohol (*c*, 0·5) [Found: OMe, 52·3; OMe, 15·6 (after saponification with barium hydroxide; equiv., 93. Calc. for $C_7H_{12}O_6$: OMe, 50·0%; equiv., 96. After saponification it would have OMe, 16·7%]. Treatment of this fraction with methyl-alcoholic methylamine gave the crystalline bismethylamide of 3-hydroxy-2-methoxyerythrosuccinic acid, m. p. 136°, $[\alpha]_D^{18°}$ +10° in water (*c*, 1·0) (after recrystallisation from ethyl acetate). It gave no depression of the m. p. when mixed with that prepared above. Fraction II (0·3 g.), b. p. (bath temp.) 140—150°/0·02 mm., $n_D^{18°}$ 1·4530. This fraction failed to give a crystalline bismethylamide, but upon treatment with methyl-alcoholic ammonia it yielded the crystalline diamide of trimethyl mannosaccharic acid, m. p. and mixed m. p. with specimen obtained above 259° (decomp.) (after recrystallisation from water).

Treatment of portion E. Examination of the two formulæ (VI) and (XII) proposed originally for the 2 : 5-dimethyl Δ⁴-mannosaccharo-3 : 6-lactone 1-methyl ester shows that the former gives upon ozonisation the aldehydic acid (VII) (2-methyl *l*-erythuronic acid); upon reduction of its aldehydic group, this acid should give 2-methyl erythronic acid, the amide of which would show a negative Weerman test for α-hydroxy-amides. On the other hand, the substance having formula (XII) would give rise to 3-methyl *l*-erythronic acid, which is an α-hydroxy-amide and would be expected to give a positive Weerman test. With these considerations in mind, portion E (30 c.c.) was subjected to the action of 2½% sodium amalgam (100 g.) during 8 hours with vigorous stirring. A subsequent search for the monomethyl *l*-erythronic acid, however, was unsuccessful.

Hydrogenation of 2 : 5-Dimethyl Δ⁴-Mannosaccharo-3 : 6-lactone 1-Methyl Ester.—A solution of this ester [0·5 g. of fraction II prepared by method (C) above] in ethyl alcohol (50 c.c.) was shaken in an atmosphere of hydrogen in the presence of platinum oxide (0·2 g.) during an hour at slightly more than 1 atm. pressure. The absorption of hydrogen then appeared to be complete. The hydrogen was expelled from the reduction vessel with a stream of carbon dioxide. The solution was filtered, and evaporated to dryness under reduced pressure. The syrupy product still had an absorption band in aqueous solution at λ 2290 A., the intensity of which indicated that approximately 75% of the unsaturated compound had been reduced. In order to complete the reduction the syrup was dissolved in ethyl alcohol (60 c.c.) and shaken with hydrogen for 8 hours in the presence of a palladium–charcoal catalyst. This hydrogenation gave a neutral syrup which showed no absorption in aqueous solution (tested at 40 mg./100 c.c.). Distillation of the syrup gave: fraction I (0·1 g.), b. p. (bath temp.) 144°/0·03 mm., $n_D^{20°}$ 1·4400, which yielded the diamide of 2 : 3 : 5-trimethyl mannosaccharic acid, m. p. 257° (decomp.), $[\alpha]_D^{19°}$ −38° in water (*c*, 0·7); fraction II (0·4 g.), b. p. (bath temp.) 160°/0·03 mm., $n_D^{20°}$ 1·4524, $[\alpha]_D^{20°}$ −4° (*ca.*) in water (*c*, 1·0) [Found, by heating with sodium hydroxide: equiv., 113. $C_8H_{14}O_6$ (2 : 5-dimethyl 4-deoxymannosaccharo-3 : 6-lactone 1-methyl ester) requires equiv., 114]. Treatment of this fraction with methyl-alcoholic ammonia for 2 days at −5° gave the *diamide* of 2 : 5-dimethyl 4-deoxy-mannosaccharic acid, which crystallised upon removal of the solvent; m. p. 187°, $[\alpha]_D^{19°}$ −74° in water (*c*, 2·6) (after crystallisation from ethyl alcohol) (Found: C, 42·5; H, 7·3; OMe, 28·15. $C_8H_{16}O_5N_2$ requires C, 42·6; H, 7·3; OMe, 28·2%).

An ice-cold solution of this diamide (20 mg.) in water (0·4 c.c.) was treated with a solution of 1·5N-sodium hypochlorite (0·2 c.c.) for 30 mins. at 0°. Removal of the excess of the sodium hypochlorite was effected by careful addition of a few drops of a fairly concentrated solution of sodium thiosulphate (tested with acidified starch–iodide paper), and this was followed by the addition of solid sodium acetate (0·2 g.) and semicarbazide hydrochloride (0·04 mg.). No hydrazodicarbonamide was obtained. A control experiment carried out under the same conditions with gluconamide (20 mg.) readily gave a positive Weerman test, indicated by the ready formation of hydrazodicarbonamide (m. p. and mixed m. p. 258°) in good yield within a minute of the addition of the semicarbazide hydrochloride.

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57. *Synthesis of the Bismethylamides of 2-Hydroxy-3-methoxy-*d*- and -*l*-erythrosuccinic Acid.*

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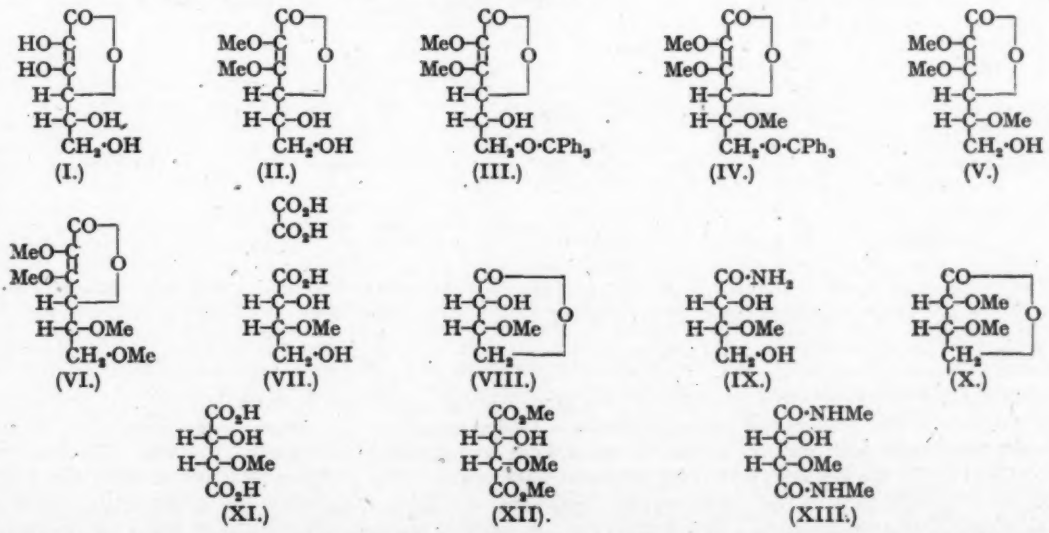
Methylation of mannosaccharodilactone either with diazomethane or with Purdie's reagents gives mainly 2 : 5-dimethyl Δ^4-mannosaccharo-3 : 6-lactone 1-methyl ester. The proof of the structure of this compound depended upon its transformation by ozonolysis and oxidation into a hitherto unknown hydroxymethoxyerythrosuccinic acid (see preceding paper). In order to determine the structure of the 2 : 5-dimethyl Δ^4-mannosaccharo-3 : 6-lactone 1-methyl ester it was necessary to establish the stereochemical configuration of the new hydroxymethoxyerythrosuccinic acid. This was achieved by the synthesis of the bismethylamides of the two enantiomorphic hydroxymethoxyerythrosuccinic acids.

The bismethylamide of 3-hydroxy-2-methoxy-*l*-erythrosuccinic acid (XIII), which may also be designated equally well as 2-hydroxy-3-methoxy-*d*-erythrosuccinic acid, was obtained from *d*-araboascorbic acid (I). The latter was transformed by successive stages into 2 : 3-dimethyl *d*-araboascorbic acid (II), 6-*trityl* 2 : 3-*dimethyl* d-*araboascorbic acid* (III), 6-*trityl* 2 : 3 : 5-*trimethyl* d-*araboascorbic acid* (IV), and thence into 2 : 3 : 5-*trimethyl* d-*araboascorbic acid* (V). Ozonisation of the last yielded 3-*methyl* d-*erythronic acid* (VII), which readily afforded a very stable *γ-lactone* (VIII). Oxidation of this lactone with nitric acid gave 2-hydroxy-3-methoxy-*d*-erythrosuccinic acid (or 3-hydroxy-2-methoxy-*l*-erythrosuccinic acid) (XI) and this, after esterification and treatment with methylamine, gave rise to the crystalline bismethylamide of 2-hydroxy-3-methoxy-*d*-erythrosuccinic acid (or 3-hydroxy-2-methoxy-*l*-erythrosuccinic acid) (XIII), m. p. 136°, $[\alpha]_D$ + 11° in water.

The *bismethylamide* of 3-hydroxy-2-methoxy-*d*-erythrosuccinic acid (XXIII), which may also be referred to as 2-hydroxy-3-methoxy-*l*-erythrosuccinic acid, was obtained from *meso*-tartaric acid (*i.e.*, dihydroxyerythrosuccinic acid). The latter was converted by means of methyl sulphate and sodium hydroxide solution into the racemic mixture of the hydroxymethoxyerythrosuccinic acids, which was resolved by means of brucine. The less water-soluble brucine salt afforded, upon treatment with alkali, followed by acid, 3-hydroxy-2-methoxy-*d*-erythrosuccinic acid (XXI), which after esterification, followed by treatment with methylamine, yielded the crystalline bismethylamide of 3-hydroxy-2-methoxy-*d*-erythrosuccinic acid (or 2-hydroxy-3-methoxy-*l*-erythrosuccinic acid) (XXIII), m. p. 135°, $[\alpha]_D$ −10·5° in water.

The bismethylamide of 2-hydroxy-3-methoxy-*d*-erythrosuccinic acid was obtained during the course of an investigation upon *d*-araboascorbic acid (I), which was obtained from methyl 2-keto-*d*-gluconate by the action of sodium methoxide (Maurer and Scheidt, *Ber.*, 1933, **66**, 1054). When *d*-araboascorbic acid (I) was allowed to react with an excess of ethereal diazomethane, the enolic hydroxy-groups in positions 2 and 3 underwent methylation, giving 2 : 3-dimethyl *d*-araboascorbic acid (II) (Hawkins, Hirst, and Jones, J., 1939, 246). Treatment of (II) in anhydrous pyridine with trityl chloride gave 6-*trityl* 2 : 3-*dimethyl* d-*araboascorbic acid* (III) and this upon complete methylation with silver oxide and methyl iodide yielded 6-*trityl* 2 : 3 : 5-*trimethyl* d-*araboascorbic acid* (IV). Saturation of a chloroform solution of the latter with hydrogen chloride readily removed the trityl group with the formation of 2 : 3 : 5-*trimethyl* d-*araboascorbic acid* (V), which yielded upon methylation with silver oxide and methyl iodide crystalline 2 : 3 : 5 : 6-*tetramethyl* d-*araboascorbic acid* (VI). The relationship of the 2 : 3-dimethyl (II), the 2 : 3 : 5-trimethyl (V) and the 2 : 3 : 5 : 6-tetramethyl *d*-araboascorbic acid (VI) is shown by the observation that all these substances exhibit an intense selective absorption band at λ 2350 A., as do the corresponding methyl derivatives of *l*-ascorbic acid (Haworth, Hirst, and Smith, *loc. cit.*; Haworth, Hirst, Smith, and Wilson, J., 1937, 829).



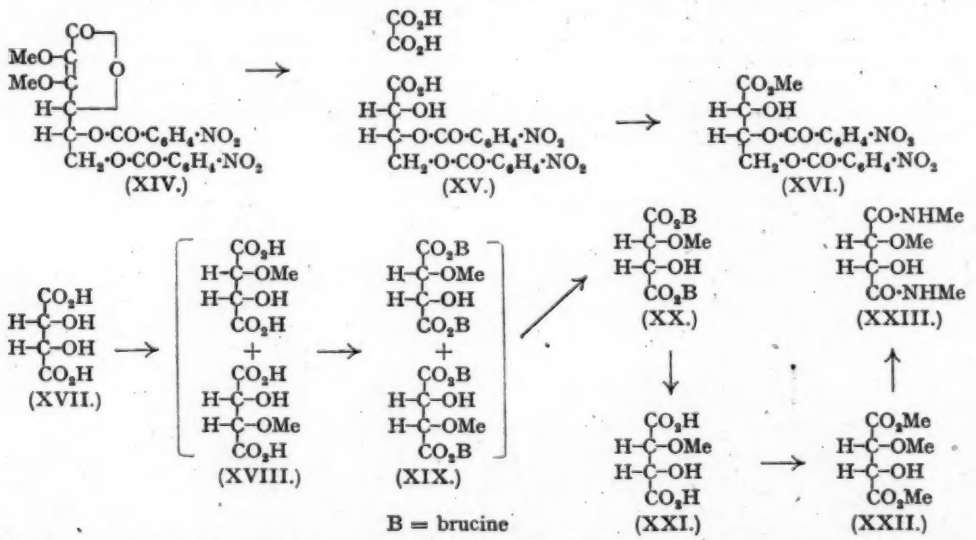
Ozonisation of 2 : 3 : 5-trimethyl *d*-araboascorbic acid (V), followed by hydrolysis, caused disruption of the double bond with the formation of oxalic acid and 3-methyl *d*-erythronic acid (VII), which upon esterification and subsequent distillation yielded crystalline 3-*methyl* d-*erythronolactone* (VIII). The presence of

the γ-lactone ring in the latter was supported by the fact that it showed no appreciable mutarotation in aqueous solution even after several months and reacted slowly in the cold with one equivalent proportion of sodium hydroxide. The 3-methyl *d*-erythronolactone (VIII) was characterised by its transformation into a crystalline *amide* (IX) and a crystalline *methylamide*. The positive Weerman test for α-hydroxy-amides (*Rev. Trav. chim.*, **1917**, **36**, 16) shown by the amide (IX) proved that there must be a free hydroxy-group at C_2. The single methyl group is therefore located in position 3. These results lend strong support to the structure (I) assigned previously to *d*-araboascorbic acid by analogy with that proved for *l*-ascorbic acid (Baird, Haworth, Herbert, Hirst, Smith, and Stacey, J., **1934**, **62**; Herbert, Hirst, Percival, Reynolds, and Smith, J., **1933**, 1270). Further methylation of 3-methyl γ-*d*-erythronolactone (VIII) with silver oxide and methyl iodide gave 2 : 3-dimethyl γ-*d*-erythronolactone (X), characterised by its conversion into a crystalline *amide*.

The crystalline 3-methyl γ-*d*-erythronolactone (VIII) proved to be relatively stable to oxidation by nitric acid, probably because of the stability of the γ-lactone ring. Prolonged treatment with nitric acid, however, gave the corresponding dibasic acid (XI), from which the methyl ester (XII) was obtained by the action of either methyl-alcoholic hydrogen chloride or ethereal diazomethane. When this dimethyl ester (XII) was treated with methyl-alcoholic methylamine, the crystalline *bismethylamide* (XIII) of 2-hydroxy-3-methoxy-*d*-erythro-succinic acid (m. p. 136°; $[\alpha]_D$ + 11° in water) was obtained. This bismethylamide (XIII) was identical with that produced by the degradation of 2 : 5-dimethyl Δ^4-mannosaccharolactone methyl ester (see previous paper).

An attempt was then made to prepare the enantiomorph of 2-hydroxy-3-methoxy-*d*-erythrosuccinic acid, namely, 2-hydroxy-3-methoxy-*l*-erythrosuccinic acid, which is the same as 3-hydroxy-2-methoxy-*d*-erythro-succinic acid, from 5 : 6-*di*-p-*nitrobenzoyl* 2 : 3-*dimethyl* d-*araboascorbic acid* (XIV) as follows. Ozonisation of (XIV) in glacial acetic acid afforded a stable ozonide, which upon decomposition gave rise to oxalic acid and 3 : 4-di-*p*-nitrobenzoyl *d*-erythronic acid (XV), characterised as its crystalline *methyl* ester (XVI). It was expected that methylation of this methyl ester (XVI), followed by elimination of the *p*-nitrobenzoyl groups, would lead to the production of methyl 2-methoxy-*d*-erythronate and that this upon oxidation would give the required 3-hydroxy-2-methoxy-*d*-erythrosuccinic acid (XXI), the enantiomorph of 2-hydroxy-3-methoxy-*d*-erythrosuccinic acid. This scheme failed, however, because of the ease with which the *p*-nitrobenzoyl groups were split off from the methyl ester of 3 : 4-di-*p*-nitrobenzoyl *d*-erythronic acid (XVI) when it was methylated either with silver oxide and methyl iodide or with diazomethane. The ready removal of the *p*-nitrobenzoyl groups is probably dependent upon the weakening of the ester link caused by the electronic influence of the *p*-nitrophenyl group (Kindler, *Annalen*, **1926**, **450**, 1; **1927**, **452**, 90).

A successful isolation of 3-hydroxy-2-methoxy-*d*-erythrosuccinic acid (XXI) was achieved, however, by resolution of the *dl*-mixture of the monomethyl derivatives of *meso*-tartaric acid by means of brucine. The



meso-tartaric acid (2 : 3-dihydroxyerythrosuccinic acid) (XVII), obtained from maleic acid by the action of sodium chlorate in the presence of osmium tetroxide (Miles and Terry, *J. Amer. Chem. Soc.*, **1925**, **47**, 1415), gave upon treatment with methyl sulphate and sodium hydroxide (cf. Haworth, J., **1915**, **107**, 15), the *dl*-mixture (XVIII) of the hydroxymethoxyerythrosuccinic acids. This racemic mixture of acids (XVIII) was purified by crystallisation of the corresponding diamides, and from the *dl*-mixture of the latter the racemic mixture of acids was regenerated. The mixture of the neutral brucine salts (XIX) of the hydroxymethoxy-erythrosuccinic acids readily crystallised and repeated fractional crystallisation afforded the less soluble brucine salt (XX) of 3-hydroxy-2-methoxy-*d*-erythrosuccinic acid. Treatment of this brucine salt with barium hydroxide yielded the barium salt of 3-hydroxy-2-methoxy-*d*-erythrosuccinic acid (XXI) and this upon boiling

with methyl-alcoholic hydrogen chloride gave methyl 3-hydroxy-2-methoxy-*d*-erythrosuccinate (XXII). When the latter was allowed to react with methyl-alcoholic methylamine, there was obtained the required crystalline *bismethylamide* (XXIII) of 3-hydroxy-2-methoxy-*d*-erythrosuccinic acid (m. p. 135°; $[\alpha]_D$ − 10·5° in water), the enantiomorph of (XIII).

Experimental.

d-*Araboascorbic Acid* (I).—To a boiling solution of methyl 2-keto-*d*-gluconate (26 g.) in dry methyl alcohol (100 c.c.), through which a stream of nitrogen was bubbled, there was added with shaking a dry methyl-alcoholic solution (80 c.c.) of sodium methoxide (1 equiv.). The boiling was continued for 1—2 minutes and most of the precipitate of sodium *d*-araboascorbate appeared to have separated. The solution was cooled in ice and the sodium *d*-araboascorbate was converted into *d*-araboascorbic acid by the addition of methyl-alcoholic hydrogen chloride (1 equiv.). The solution was filtered, and evaporated to dryness under diminished pressure in an atmosphere of carbon dioxide, and the solid residue was extracted with ethyl alcohol until the insoluble sodium chloride was free from *d*-araboascorbic acid (tested with iodine and with Fehling's solution). Concentration of the filtrate gave *d*-araboascorbic acid (16 g.), m. p. and mixed m. p. 169°, $[\alpha]_D^{18°}$ − 14° in water (*c*, 0·8), after recrystallisation from ethyl alcohol (15·6 mg. reacted with 9·1 c.c. of N/50-iodine, corresponding to M = 172) (Found: C, 41·0; H, 4·2. Calc. for $C_6H_8O_6$: C, 40·8; H, 4·6%). Aqueous solutions of *d*-araboascorbic acid showed an intense band at λ *ca.* 2450 A., ε approx. 7500 (*c*, 3·2 mg. %).

2 : 3-*Dimethyl* d-*Araboascorbic Acid* (II).—A solution of *d*-araboascorbic acid (16 g.) in a small volume of dry methyl alcohol was treated for 2 days at − 5° with a slight excess of an ethereal solution of diazomethane. The volume of methyl alcohol should be sufficient to prevent precipitation of the *d*-araboascorbic acid when the ethereal solution of diazomethane is added. The persistent yellow colour of the solution, after evolution of nitrogen had ceased, indicated that an excess of diazomethane was present. Removal of a small amount of flocculent precipitate from the solution by filtration, followed by evaporation to dryness under diminished pressure, gave syrupy 2 : 3-dimethyl *d*-araboascorbic acid (18 g.), $[\alpha]_D^{20°}$ − 14° in water (*c*, 1·0) (Found: OMe, 33·0. Calc. for $C_8H_{12}O_6$: OMe, 30·4%). In aqueous solution 2 : 3-dimethyl *d*-araboascorbic acid shows a band at λ 2350 A., ε approx. 7000 (*c*, 4·0 mg./100 c.c.), disappearing upon the addition of a slight excess of sodium hydroxide.

6-*Trityl* 2 : 3-*Dimethyl* d-*Araboascorbic Acid* (III).—A solution of the syrupy 2 : 3-dimethyl *d*-araboascorbic acid (16·4 g.) in anhydrous pyridine (20 c.c.) was treated with triphenylmethyl chloride (25 g.) at room temperature for 5 days. After the reaction had been proceeding for 2 days the mixture was warmed for 5 minutes on the hot water-bath and this was again repeated on the third day. On the fifth day the reaction mixture was poured into water and the insoluble material was triturated to remove as much pyridine as possible. After purification by recrystallisation from methyl alcohol, followed by crystallisation from acetone–light petroleum, the 6-*trityl* 2 : 3-*dimethyl* d-*araboascorbic acid* had m. p. 174°, $[\alpha]_D^{18°}$ − 41° in chloroform (*c*, 1·0) (Found: C, 72·3; H, 6·85; OMe, 14·2. $C_{27}H_{28}O_6$ requires C, 72·5; H, 6·8; OMe, 13·9%). The crystals tend to retain methyl alcohol. Unlike the 6-trityl 2 : 3-dimethyl *l*-araboascorbic acid, this 6-trityl 2 : 3-dimethyl *d*-araboascorbic acid was not affected by treatment with methyl-alcoholic ammonia (cf. Micheel, *Annalen*, 1935, **510**, 70; Haworth, Hirst, Smith, and Wilson, *loc. cit.*).

6-*Trityl* 2 : 3 : 5-*Trimethyl* d-*Araboascorbic Acid* (IV).—Three treatments of 6-trityl 2 : 3-dimethyl *d*-araboascorbic acid (2 g.) with silver oxide and methyl iodide in the usual manner effected complete methylation of the compound. The methyl iodide was distilled off, and the product extracted with acetone. Removal of the solvent gave 6-*trityl* 2 : 3 : 5-*trimethyl* d-*araboascorbic acid* (2·0 g.) as a glass, $[\alpha]_D^{18°}$ − 28° in chloroform (*c*, 1·0) (Found: OMe, 20·4. $C_{28}H_{28}O_6$ requires OMe, 20·2%).

2 : 3 : 5-*Trimethyl* d-*Araboascorbic Acid* (V).—A solution of 6-trityl 2 : 3 : 5-trimethyl *d*-araboascorbic acid (12·0 g.) in a mixture of chloroform (200 c.c.) and methyl alcohol (10 c.c.) was cooled in an ice-bath and saturated with dry hydrogen chloride. After keeping for 1 hour at 0°, the ice-bath was removed and after a further 2 hours as much hydrogen chloride as possible was removed from the solution by aeration under diminished pressure. The chloroform solution was then exhaustively extracted with water; the aqueous extracts were combined, neutralised with lead carbonate, filtered, and evaporated to dryness under reduced pressure. The syrupy product was purified by extraction with acetone (yield, 3·8 g.) (Found: OMe, 41·0%). The product distilled as a colourless oil (3·0 g.), b. p. (bath temp.) 170°/0·02 mm., $n_D^{18°}$ 1·4880, $[\alpha]_D^{18°}$ + 9° in water (*c*, 1·0), absorption band at λ 2350 A. (ε, 10,000) (*c*, 3·4 mg. % in water) (Found: OMe, 42·3%). On cooling, the distillate crystallised; the crystals were separated by tiling and after recrystallisation from dry ether the 2 : 3 : 5-*trimethyl* d-*araboascorbic acid* had m. p. 74°, $[\alpha]_D^{22°}$ + 10° in water (*c*, 0·8) (Found: C, 49·6; H, 6·5; OMe, 43·1. $C_9H_{14}O_6$ requires C, 49·6; H, 6·4; OMe, 42·6%). Aqueous solutions of the crystalline 2 : 3 : 5-trimethyl *d*-araboascorbic acid show an intense band at λ 2350 A., ε approx. 11,000 (*c*, 3 mg. %).

2 : 3 : 5 : 6-*Tetramethyl* d-*Araboascorbic Acid* (VI).—Two methylations of crystalline 2 : 3 : 5-trimethyl *d*-araboascorbic acid (0·45 g.) with silver oxide (1 g.) and methyl iodide (3 c.c.) were sufficient to introduce the methoxyl group into position 6. After each methylation the product was isolated by means of acetone. The compound distilled as a colourless liquid (0·4 g.), b. p. (bath temp.) 130°/0·02 mm., $n_D^{22°}$ 1·4695, $[\alpha]_D^{20°}$ + 9·5° in water (*c*, 0·5), absorption band at λ 2350 A., ε approx. 10,000 (*c*, 3 mg.% in water) (Found: OMe, 53·0. $C_{10}H_{16}O_6$ requires OMe, 53·4%).

Ozonisation of 2 : 3 : 5-*Trimethyl* d-*Araboascorbic Acid*.—A solution of the acid (3·0 g.) in glacial acetic acid (30 c.c.) was subjected to the action of a stream of ozonised oxygen at room temperature. The solution showed $[\alpha]_D^{18°}$ − 1° (initial value); − 2·5° (after 3 hours); + 1° (6 hours); + 8° (8 hours); + 30° (12 hours); + 41° (14 hours); + 52° (17 hours). The solution was diluted with water, freed from water and acetic acid by evaporation under reduced pressure, and neutralised with barium hydroxide, and the barium oxalate (85% of the theoretical) removed. The filtrate containing the soluble barium salt of 3-methyl *d*-erythronic acid was evaporated under diminished pressure at 40°, and the dry residue (3·14 g.) boiled with methyl-alcoholic hydrogen chloride (200 c.c.). Sufficient hydrogen chloride was used to liberate the free acid from the barium salt and to give a solution of 1% hydrogen chloride with which to effect esterification, which was brought about by boiling the solution for 8 hours. The solution was neutralised with silver carbonate, filtered, and evaporated under reduced pressure to dryness. The product was purified by extraction with ether to remove inorganic impurity and then distilled, giving fraction I (0·6 g.), b. p. (bath temp.) 152°/0·04 mm., $n_D^{18°}$ 1·4565—1·4572, fraction II (0·51 g.), b. p. (bath temp. 200—210°/0·05 mm., $n_D^{18°}$ 1·4700.

Examination of fraction I. Isolation of 3-*methyl* d-*erythronolactone* (VIII). This fraction crystallised spontaneously and after 1 hour the crystals were freed from adhering syrup by trituration with ethyl alcohol–ether. After two recrystallisations from ethyl alcohol–ether the 3-*methyl* d-*erythronolactone* (0·1 g.) had m. p. 113°, $[\alpha]_D^{19°}$ − 108° in water (*c*, 4·0). No appreciable change in rotation took place after this solution had been kept for several months. The lactone reacted slowly when titrated directly with 0·01N-sodium hydroxide; on being kept for 1 hour with an excess of 0·01N-sodium hydroxide in the cold, followed by back-titration with 0·01N-sulphuric acid, one equivalent proportion was taken up (8·19 mg. required for neutralisation 7·14 c.c. of 0·01N-sodium hydroxide, corresponding to an equivalent weight of 127. Calc., 131) (Found: C, 45·1; H, 6·5; OMe, 23·4. $C_5H_8O_4$ requires C, 45·4; H, 6·1; OMe, 23·5%).

Treatment of the crystalline 3-methyl *d*-erythronolactone with methyl-alcoholic ammonia furnished the crystalline

amide (IX) of 3-methyl *d*-erythronic acid, which after recrystallisation from ethyl alcohol had m. p. 105°, $[\alpha]_D^{18°}$ + 36° in water (*c*, 1·0) (Found: C, 40·9; H, 7·6; N, 9·4; OMe, 20·6. $C_5H_{11}O_4N$ requires C, 40·3; H, 7·4; N, 9·4; OMe, 20·8%). When this amide (20 mg.) was treated for 30 minutes with 1·5N-sodium hypochlorite under the standard conditions for a Weerman reaction, it gave a positive test (see Smith, J., 1939, 753), because upon the addition of excess of solid sodium acetate and semicarbazide hydrochloride a precipitate of hydrazodicarbonamide, m. p. and mixed m. p. 254°, was obtained.

With methyl-alcoholic methylamine the 3-methyl *d*-erythronolactone gave the crystalline *methylamide* of 3-methyl *d*-erythronic acid, m. p. 82°, $[\alpha]_D^{17°}$ + 57·5° in methyl alcohol (*c*, 2·1) (Found: C, 44·5; H, 8·2; N, 8·6; OMe, 18·3. $C_6H_{13}O_4N$ requires C, 44·2; H, 8·0; N, 8·6; OMe, 19·0%).

Methylation of 3-*Methyl* d-*Erythronolactone*.—The crystalline 3-methyl *d*-erythronolactone (100 mg.) was dissolved in methyl iodide (3 c.c.) and refluxed for 7 hours in the presence of silver oxide (1 g.) with frequent shaking. The product was isolated by means of acetone and subjected to a second methylation in the same way. The product, a liquid which failed to crystallise after several days, was treated with methyl-alcoholic ammonia for 2 days at − 5°. Removal of the solvent gave the *amide* of 2 : 3-dimethyl *d*-erythronic acid, m. p. 72°, $[\alpha]_D^{21°}$ + 55·5° in water (*c*, 1·8), after recrystallisation from acetone–ether (Found: OMe, 38·3; N, 9·5. $C_6H_{13}O_4N$ requires OMe, 38·2; N, 9·4%).

Oxidation of 3-*Methyl* d-*Erythronolactone with Nitric Acid and the Isolation of the Bismethylamide of* 2-*Hydroxy*-3-*methoxy*-d-*erythrosuccinic Acid* (XIII).—Concentration of the mother-liquors from the crystallisation of the crystalline 3-methyl *d*-erythronolactone gave a syrupy crystalline product (0·5 g.). This was treated for 24 hours at 50° with nitric acid (10 c.c. of a solution made by mixing equal volumes of nitric acid, *d* 1·42, and water). The solution was then diluted with water, and the solvent removed by distillation under diminished pressure, methyl alcohol being added in the final stages to decompose any remaining traces of nitric acid. The dry syrupy acidic product was then boiled for 8 hours with 1% methyl-alcoholic hydrogen chloride (20 c.c.). Neutralisation of the mineral acid with silver carbonate, followed by removal of the solvent, gave a syrup, which was purified by extraction with ether and then distilled giving fraction I (0·1 g.), b. p. (bath temp.) 130°/0·04 mm., $n_D^{19°}$ 1·4466, $[\alpha]_D^{18°}$ − 34° in methyl alcohol (*c*, 2·0) (Found: OMe, 46·5; equiv., 97. $C_7H_{12}O_6$ requires OMe, 48·4%; equiv., 96), and fraction II, b. p. (bath temp.) 160°/0·04 mm., $n_D^{19°}$ 1·4560. This fraction readily crystallised and gave the unchanged lactone, m. p. and mixed m. p. 113° (after recrystallisation from ethyl alcohol).

Treatment of fraction I (40 mg.) with methyl-alcoholic methylamine for 24 hours at room temperature, followed by removal of the solvent, yielded the *bismethylamide* of 2-hydroxy-3-methoxy-*d*-erythrosuccinic acid, m. p. 136°, $[\alpha]_D^{18°}$ +11° in water (*c*, 2·5) (after recrystallisation from ethyl acetate). This bismethylamide gave no depression when mixed with that obtained from 2 : 5-dimethyl Δ^4-mannosaccharolactone methyl ester (previous paper) (Found: C, 44·3; H, 6·8; N, 14·8; OMe, 16·2. $C_7H_{14}O_4N_2$ requires C, 44·2; H, 7·4; N, 14·7; OMe, 16·3%).

In another experiment pure crystalline 3-methyl *d*-erythronolactone (0·1 g.) was oxidised for 48 hours at 60° with nitric acid (4 c.c. of a solution made by mixing equal volumes of nitric acid, *d* 1·42, and water). The solution was diluted with water and the product was freed from solvent and nitric acid as described above. In this way there was obtained a syrupy crystalline mass; the crystals and syrup were separated by means of alcohol. The crystals proved to be unchanged 3-methyl *d*-erythronolactone, m. p. and mixed m. p. 113°. The crystals and syrup were recombined and subjected to oxidation with the same amount of nitric acid for 5 hours at 90°. This reaction gave unchanged crystalline lactone (60 mg.) and a syrup (30 mg.) which reacted strongly acid to Congo-paper. This syrupy acid was dissolved in ether and esterified by treatment with a slight excess of an ethereal solution of diazomethane for 5 minutes at 0°. Removal of the solvent gave a neutral syrup which, when treated with methyl-alcoholic methylamine for 24 hours at room temperature, afforded the crystalline bismethylamide of 2-hydroxy-3-methoxy-*d*-erythrosuccinic acid (15 mg.), m. p. 135°, $[\alpha]_D^{18°}$ + 11° in water (*c*, 4·5) (after recrystallisation from ethyl acetate).

5 : 6-*Di*-p-*nitrobenzoyl* 2 : 3-*Dimethyl* d-*Araboascorbic Acid* (XIV).—A solution of 2 : 3-dimethyl *d*-araboascorbic acid (2·5 g.) in dry pyridine (5 c.c.) was allowed to react with *p*-nitrobenzoyl chloride (2·3 g.) for 48 hours at room temperature. The mixture was then warmed for 5 minutes at 60° in order to complete the reaction, cooled, and poured with stirring into water. The *p*-nitrobenzoyl derivative was extracted with chloroform, and the chloroform solution repeatedly washed with sodium bicarbonate solution to remove *p*-nitrobenzoic acid. After drying over anhydrous magnesium sulphate, the chloroform solution was evaporated to dryness under reduced pressure and there was obtained 5 : 6-*di*-p-*nitrobenzoyl* 2 : 3-*dimethyl* d-*araboascorbic acid* (4·2 g.) as a glass (Found: OMe, 8·9. $C_{22}H_{20}O_{12}N_2$ requires OMe, 8·9%).

Methyl 3 : 4-*Di*-p-*nitrobenzoyl* d-*Erythronate* (XVI).—A stream of ozonised oxygen was passed through a solution of the 5 : 6-di-*p*-nitrobenzoyl 2 : 3-dimethyl *d*-araboascorbic acid (4 g.) in glacial acetic acid (50 c.c.) for 15 hours at room temperature; the specific rotation changed from +15° to +23°. The presence of oxalate in the reaction mixture after 15 hours' ozonolysis could not be detected. The solution was diluted with water and freed from solvent by evaporation under diminished pressure. Methyl alcohol was added from time to time to the product during the last stages of the distillation to remove traces of acetic acid. The syrupy product thus obtained crystallised on keeping. After recrystallisation from aqueous ethyl alcohol, the *methyl* 3 : 4-*di*-p-*nitrobenzoyl* d-*erythronate* had m. p. 133°, $[\alpha]_D^{17°}$ + 29° in chloroform (*c*, 1·3) (Found: C, 50·9; H, 3·6; N, 6·4; OMe, 6·8. $C_{19}H_{16}O_{11}N_2$ requires C, 50·9; H, 3·6; N, 6·2; OMe, 6·9%).

Methylation of the methyl 3 : 4-di-*p*-nitrobenzoyl *d*-erythronate either with Purdie's reagents or with diazomethane effected the elimination of the *p*-nitrobenzoyl groups as methyl *p*-nitrobenzoate, m. p. and mixed m. p. 92° (after recrystallisation from ethyl alcohol).

Methylation of meso-*Tartaric Acid*.—*meso*-Tartaric acid (10 g.), prepared by the method of Miles and Terry (*J. Amer. Chem. Soc.*, 1925, **47**, 1415), was dissolved in sodium hydroxide (100 c.c. of a 30% solution) at 55°. Methyl sulphate (100 c.c.) and sodium hydroxide (200 c.c. of a 30% solution) were then added slowly during 3 hours to the solution at 55—60° with vigorous stirring. The reaction mixture was heated for 30 minutes at 90°, cooled, and made acid by the addition of 5N-sulphuric acid. The cold solution was filtered to remove sodium sulphate and, while still acid, was distilled under reduced pressure for a sufficient length of time to remove all carbon dioxide. It was then neutralised with sodium hydroxide, treated with 5N-sulphuric acid (20 c.c.) in order to liberate 60% of the organic acid, and concentrated to dryness under diminished pressure, and the organic acid extracted with acetone–ether. Removal of the solvent gave an acidic syrup, which was freed from a little sodium sulphate by extraction with ether. From the ethereal solution there was obtained an acidic syrup (4 g.). Further treatment of an aqueous solution of the residues of the sodium salt with more 5N-sulphuric acid (10 c.c.), followed by evaporation to dryness and extraction with acetone–ether, gave a further amount of the crude syrupy monomethyl derivative (2 g.).

The syrupy mixture (6 g.) of the monomethyl derivatives of *meso*-tartaric acid was esterified by boiling for 8 hours with 1% methyl-alcoholic hydrogen chloride (300 c.c.). The solution was cooled, neutralised with barium carbonate, filtered, and evaporated to dryness. The residue was extracted with acetone–ether and upon removal of the solvent there was obtained a mixture of syrup and crystals. The latter were separated by trituration with ether and after recrystallisation from acetone–ether the crystals (0·6 g.) had m. p. 114° alone or when mixed with methyl *meso*-tartrate. Concentration of the ethereal solution, after separation of the crystalline methyl *meso*-tartrate, yielded a syrup, which

was distilled, giving: Fraction I (a racemic mixture of the two forms of hydroxymethoxyerythrosuccinic acid) (2·6 g.), b. p. (bath temp.) 100—105°/0·04 mm., $n_D^{18°}$ 1·4400. Fraction II, methyl *meso*-tartrate (1·8 g.), b. p. (bath temp.) 130—140°/0·04 mm., $n_D^{18°}$ 1·4440, m. p. 114° (after crystallisation from acetone–ether).

Treatment of fraction I with methyl-alcoholic ammonia for 3 days at −5° gave the amide of the racemic hydroxymethoxyerythrosuccinic acid, m. p. 191° (after two recrystallisations from water). The free acid was then regenerated by heating the amide (2·3 g.) with N-sodium hydroxide (30 c.c.) for 45 minutes at 60°. Addition of N-sulphuric acid (198 c.c.), followed by removal of the solvent and extraction of the dry residue with acetone–ether, gave a syrupy racemic mixture (XVIII) of the hydroxymethoxyerythrosuccinic acids (2·3 g.).

Resolution of the Racemic Mixture of Hydroxymethoxyerythrosuccinic Acids.—An attempt to resolve the racemic acids by means of the half cinchonine salt had to be abandoned because the salt failed to crystallise.

The acid was therefore converted into the neutral brucine salt by adding an alcoholic solution of brucine to a solution of the hydroxymethoxyerythrosuccinic acid (2·1 g.) in ethyl alcohol (15 c.c.). Removal of the solvent gave a crystalline brucine salt, $[\alpha]_D^{18°}$ − 23° in water (*c*, 0·7) (after recrystallisation from water).

A further quantity of *meso*-tartaric acids (15 g.) was methylated as described above, methyl sulphate (100 c.c.) and sodium hydroxide (300 c.c. of a 30% solution) being used. Esterification of the partially methylated acid afforded an ester (6·0 g.), which was distilled, giving: Fraction I (0·47 g.), methyl dimethyl *meso*-tartrate, b. p. (bath temp.) 80—95°/0·01 mm., $n_D^{19°}$ 1·4390, recognised by its transformation into the crystalline bismethylamide, m. p. and mixed m. p. 202°. Fraction II, racemic methyl hydroxymethoxyerythrosuccinate (3·63 g.), b. p. (bath temp.) 96—98°/0·01 mm., $n_D^{19°}$ 1·4410. Fraction III, methyl *meso*-tartrate (1·85 g.), b. p. 130—140°/0·02 mm., $n_D^{19°}$ 1·4440, m. p. 114° (after crystallisation from acetone–ether).

Fraction II was treated with N-sodium hydroxide (50 c.c.) for 45 minutes at 60°; N-sulphuric acid (49·5 c.c.) was added, and the solution evaporated to dryness, giving the syrupy racemic mixture of the hydroxymethoxyerythrosuccinic acids. This acid was dissolved in ethyl alcohol and titrated with an alcoholic solution of brucine. Removal of the solvent yielded the crystalline brucine salt, $[\alpha]_D^{18°}$ − 23° in water (*c*, 1·0).

Fractional Crystallisation of the Brucine Salt of Racemic Hydroxymethoxyerythrosuccinic Acid.—One recrystallisation of the brucine salt (20 g.) from water gave the less soluble portion, $[\alpha]_D^{18°}$ − 22° in water. Concentration of the mother-liquors gave crystals, $[\alpha]_D^{17°}$ − 23° in water. Four more crystallisations of this, the more soluble fraction, gave crystals (4 g.), $[\alpha]_D^{18°}$ − 26° in water (*c*, 1·7), and no alteration of rotation occurred upon further crystallisation from water. Similarly, six crystallisations of the less soluble fraction of the brucine salt gave crystals, $[\alpha]_D^{17°}$ − 17° in water (*c*, 0·7).

3-Hydroxy-2-methoxy-d-erythrosuccinobismethylamide (XXIII).—A solution of the brucine salt (1·56 g.), $[\alpha]_D^{17°}$ − 17° in water) (15 c.c.) was treated with a slight excess of a solution of barium hydroxide. The brucine was removed and the solution neutralised with carbon dioxide, filtered, and evaporated to dryness. The barium salt was then boiled for 8 hours with 2% methyl-alcoholic hydrogen chloride (150 c.c.). Neutralisation of the solution with silver carbonate, followed by removal of the solvent, gave a neutral syrupy ester, which upon treatment with methyl-alcoholic methylamine yielded the crystalline *bismethylamide* of 3-hydroxy-2-methoxy-*d*-erythrosuccinic acid, m. p. 135°, $[\alpha]_D^{20°}$ − 10·5° in water (*c*, 1·0) (after recrystallisation from ethyl acetate) (Found: C, 44·3; H, 7·45; N, 14·8; OMe, 15·9. $C_7H_{14}O_4N_2$ requires C, 44·2; H, 7·4; N, 14·75; OMe, 16·3%). This was the enantiomorph of the bismethylamide obtained from *d*-araboascorbic acid (see above) and from 2 : 5-dimethyl Δ⁴-mannosaccharolactone methyl ester (see previous paper).

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58. 3 : 6-*Anhydrogalactose. Part II. 2-Methyl and 4-Methyl* 3 : 6-*Anhydro-α-methylgalactopyranoside.*

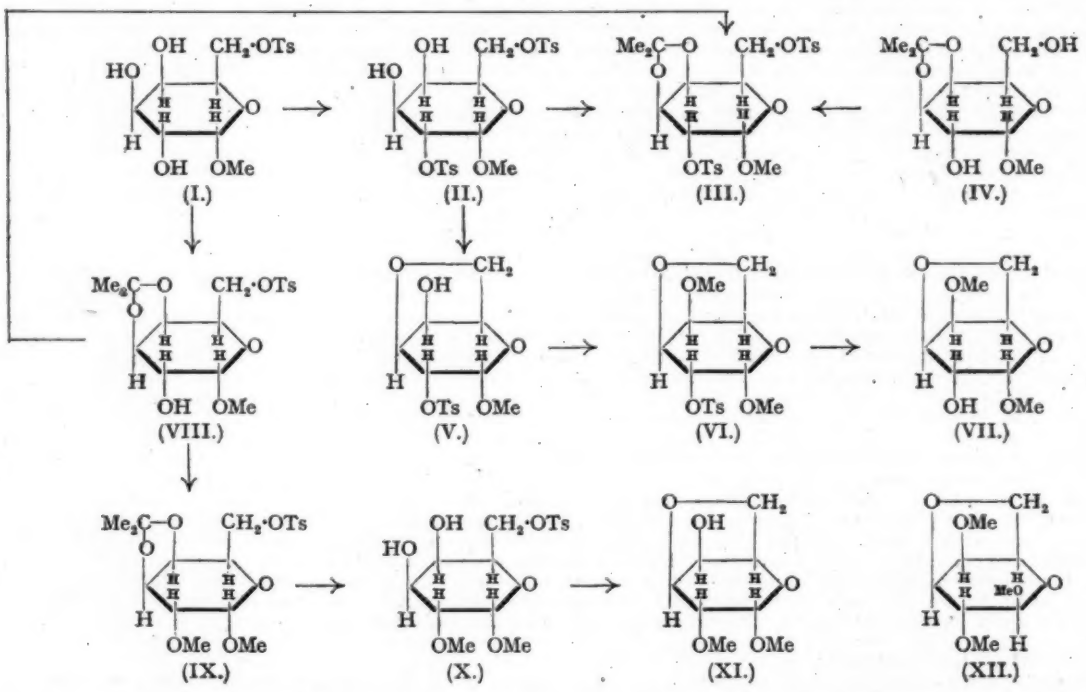
By (MRS.) P. A. RAO and F. SMITH.

The ditosyl α-methylgalactopyranoside obtained by the action of *p*-toluenesulphonyl chloride upon α-methylgalactopyranoside (Haworth, Jackson, and Smith, J., 1940, 620) has been shown to be the 2 : 6-*ditosyl* derivative (II). Mild treatment of (II) with dilute alkali yielded 2-*tosyl* 3 : 6-*anhydro-α-methylgalactopyranoside* (V), which upon methylation with silver oxide and methyl iodide afforded 2-*tosyl* 4-*methyl* 3 : 6-*anhydro-α-methylgalactopyranoside* (VI). More drastic treatment of (VI) with sodium hydroxide gave crystalline 4-*methyl* 3 : 6-*anhydro-α-methylgalactopyranoside* (VII).

Condensation of acetone with 6-tosyl α-methylgalactopyranoside (I) furnished 6-tosyl 3 : 4-monoacetone α-methylgalactoside (VIII), and this with methyl iodide and silver oxide gave 6-*tosyl* 3 : 4-*monoacetone* 2-*methyl α-methylgalactoside* (IX). Treatment of the latter with acid methyl alcohol afforded 6-*tosyl* 2-*methyl α-methylgalactopyranoside* (X), which by the agency of dilute alkali was converted into 2-*methyl* 3 : 6-*anhydro-α-methylgalactopyranoside* (XI).

IN view of the unique properties displayed by the 2 : 4-dimethyl derivatives of 3 : 6-anhydromethylgalactoside (Haworth, Jackson, and Smith, *Nature*, 1938, **142**, 1075; J., 1940, 620) it was decided to prepare for study the 2-methyl and the 4-methyl derivative of 3 : 6-anhydro-α-methylgalactopyranoside. The source of the 4-methyl derivative was the hitherto unknown ditosyl methylgalactoside, obtained by direct treatment of α-methylgalactopyranoside with *p*-toluenesulphonyl chloride in pyridine solution (Haworth, Jackson, and Smith, *loc. cit.*). The structure (II) assigned to the *ditosyl* derivative of α-methylgalactoside is based upon the following experimental facts. The ditosyl derivative can be obtained from 6-tosyl α-methylgalactopyranoside (I) by further treatment of the latter with *p*-toluenesulphonyl chloride. This demonstrates that one of the tosyl residues of (II) is attached to position 6, and it follows that the second tosyl group can occupy any one of the positions 2, 3, and 4. When the ditosyl α-methylgalactoside (II) was treated with acetone in the presence of sulphuric acid, condensation occurred with the formation of a *monoacetone ditosyl α-methylgalactoside* (III). By analogy with the behaviour of 6-tosyl α-methylgalactopyranoside (Ohle and Thiel, *Ber.*, 1933, **66**, 528) and β-methylgalactopyranoside (Bell and Williamson, J., 1938, 1198), which are known to give 3 : 4-monoacetone derivatives when condensed with acetone, it seemed probable that the acetone residue in (II)

was also attached to positions 3 and 4, in which case the second tosyl group in (III) and therefore in (II) would be located in position 2. This view was confirmed by the observation that treatment of 6-tosyl 3 : 4-monoacetone α-methylgalactopyranoside (VIII) with *p*-toluenesulphonyl chloride furnished 2 : 6-ditosyl 3 : 4-monoacetone α-methylgalactopyranoside, identical with the ditosyl monoacetone α-methylgalactoside (III) obtained from (II). It is clear, therefore, that the two tosyl groups of (II) must occupy positions 2 and 6.



When 2 : 6-ditosyl α-methylgalactoside (II) was warmed with N-aqueous-alcoholic sodium hydroxide, one tosyl group was eliminated, with the formation of 2-*tosyl* 3 : 6-*anhydro*-α-*methylgalactopyranoside* (V) in a manner analogous to the conversion of 6-tosyl α-methylgalactopyranoside into 3 : 6-anhydro-α-methylgalactoside (Ohle and Thiel, *loc. cit.*; Percival and Forbes, *Nature*, 1938, **142**, 1076; Haworth, Jackson, and Smith, *loc. cit.*). Prolonged treatment of 2 : 6-ditosyl α-methylgalactoside with 3N-sodium hydroxide results in the removal of the tosyl group from C_2 with the formation of 3 : 6-anhydro-α-methylgalactopyranoside. This observation proved, not only that the C_2 tosyl group could be removed, but also that its removal was not accompanied by a Walden inversion. Methylation of (V) with silver oxide and methyl iodide proceeded smoothly and there was produced 2-*tosyl* 4-*methyl* 3 : 6-*anhydro*-α-*methylgalactoside* (VI), from which the tosyl group in position 2 was removed without Walden inversion by more drastic treatment with aqueous alcoholic 3N-sodium hydroxide at 60°. In this way there was obtained crystalline 4-*methyl* 3 : 6-*anhydro*-α-*methylgalactopyranoside* (VII). Proof that no Walden inversion occurred at C_2 during the conversion of (VI) into (VII) was forthcoming from the fact that methylation of (VII) with silver oxide and methyl iodide yielded 2 : 4-dimethyl 3 : 6-anhydro-α-methylgalactopyranoside, which was characterised by its almost immediate transformation with hydrogen chloride into the crystalline β-form of 2 : 4-dimethyl 3 : 6-anhydro-β-methylgalactopyranoside (XII).

The 2-*methyl* 3 : 6-*anhydro*-α-*methylgalactopyranoside* (XI) was prepared by the following series of reactions which leave no doubt as to its constitution. 6-Tosyl α-methylgalactoside (I) was condensed with acetone in the presence of sulphuric acid and there was obtained 6-tosyl 3 : 4-monoacetone α-methylgalactoside (VIII). By means of silver oxide and methyl iodide this was transformed into the corresponding crystalline 6-*tosyl* 2-*methyl* 3 : 4-*monoacetone* α-*methylgalactoside* (IX). Removal of the acetone residue was easily effected with methyl-alcoholic hydrogen chloride, and there resulted 6-*tosyl* 2-*methyl* α-*methylgalactopyranoside* (X), and this upon treatment with N-aqueous-alcoholic sodium hydroxide gave the required crystalline 2-methyl α-methylgalactopyranoside (XI). That the galactose configuration still existed in the latter was proved by its conversion with silver oxide and methyl iodide into 2 : 4-dimethyl 3 : 6-anhydro-α-methylgalactoside, which under the influence of a trace of hydrogen chloride yielded crystalline 2 : 4-dimethyl 3 : 6-anhydro-β-methylgalactopyranoside (XII).

Experimental.

2 : 6-*Ditosyl* α-*Methylgalactopyranoside* (II).—(a) *From* α-*methylgalactopyranoside.* Anhydrous α-methylgalactoside, prepared from the monohydrate (5 g.) by heating in a vacuum at 110° until all water had been eliminated, was dissolved in anhydrous pyridine (15 c.c.) and treated with *p*-toluenesulphonyl chloride (2 g.) for 12 hours at room temperature and

for 2 days at 30°. The reaction mixture was warmed to 50°, then poured with stirring into water and subsequently triturated with fresh portions of water in order to remove as much pyridine as possible. Trituration with acetone at room temperature dissolved the ditosyl α-methylgalactoside and the crystalline 6-tosyl α-methylgalactoside was filtered off and washed with acetone (yield 1·5 g.), m. p. and mixed m. p. 175° (decomp.) (after recrystallisation from ethyl alcohol).

Removal of the acetone from the mother-liquors gave a syrup, which was dissolved in chloroform and the solution was extracted several times with dilute sulphuric acid to remove pyridine, with sodium bicarbonate solution (twice), water (once), and then dried over anhydrous sodium sulphate. Distillation of the solvent gave a syrup, which was dissolved in a small volume of hot ethyl alcohol. The solution was seeded with a crystal of 2 : 6-ditosyl methylgalactoside prepared in a previous experiment (Haworth, Jackson, and Smith, J., 1940, 620), and allowed to crystallise. The crystals were filtered off, washed with a little ethyl alcohol, and after recrystallisation from the same solvent the 2 : 6-ditosyl α-methylgalactopyranoside (1·0 g.) had m. p. 148°; $[\alpha]_D^{18°}$ + 68° in pyridine (*c*, 1·0).

(b) *From* 6-*tosyl* α-*methylgalactopyranoside* (I). A solution of (I) (0·5 g.) in dry pyridine (2 c.c.) was allowed to react with *p*-toluenesulphonyl chloride (0·65 g.) for 6 hours at room temperature and for 2 days at 30°. A few drops of water were stirred into the reaction mixture, and after 30 minutes the product was dissolved in chloroform (20 c.c.). The chloroform solution was extracted with dilute sulphuric acid (twice), sodium bicarbonate solution (once), water (once), and then dried over anhydrous magnesium sulphate. Removal of the solvent gave a syrup, which was dissolved in the minimum amount of ethyl alcohol. On keeping, crystallisation of the 2 : 6-ditosyl α-methylgalactoside took place. The crystals were filtered off, washed with light petroleum, and recrystallised from ethyl alcohol (yield, 0·57 g.); m. p. 148°; $[\alpha]_D^{20°}$ + 66·5° in pyridine (*c*, 0·6). 2 : 6-*Ditosyl* α-*methylgalactoside* is readily soluble in pyridine, acetone, and chloroform; it is sparingly soluble in ethyl and methyl alcohol and insoluble in light petroleum (Found : C, 49·9; H, 5·2; OMe, 6·3; S, 12·5. $C_{21}H_{28}O_{10}S_2$ requires C, 50·2; H, 5·2; OMe, 6·2; S, 12·7%).

2 : 6-*Ditosyl* 3 : 4-*Monoacetone* α-*Methylgalactopyranoside* (III).—(a) *From* 2 : 6-*ditosyl* α-*methylgalactoside* (II). When a solution of (II) (1·0 g.) in dry acetone (170 c.c.) containing concentrated sulphuric acid (0·2 c.c.) was kept at room temperature, the specific rotation changed from $[\alpha]_D^{21°}$ + 86° (initial value) to + 110° (after keeping overnight). The solution was neutralised with solid sodium bicarbonate, filtered, and evaporated to dryness. The dry residue crystallised spontaneously, and after purification by recrystallisation from ethyl alcohol the 2 : 6-*ditosyl* 3 : 4-*monoacetone* α-*methylgalactoside* had m. p. 148°; $[\alpha]_D$ + 115° in pyridine (*c*, 0·6) (Found : C, 53·2; H, 5·5; OMe, 5·8; S, 11·8. $C_{24}H_{30}O_{10}S_2$ requires C, 53·1; H, 5·5; OMe, 5·7; S, 11·8%).

(b) *From* 6-*tosyl* 3 : 4-*monoacetone* α-*methylgalactoside* (VIII). Well-powdered 6-tosyl α-methylgalactopyranoside (1 g.) was shaken with acetone (170 c.c.) containing concentrated sulphuric acid (0·2 c.c.) for 2 hours until complete solution had been attained. After remaining for a further 12 hours at room temperature, the solution was neutralised with sodium bicarbonate, filtered, and evaporated to dryness under diminished pressure. The product was purified by extraction with benzene, and after removal of the solvent, followed by crystallisation from ethyl alcohol, the 6-tosyl 3 : 4-monoacetone α-methylgalactoside (0·75 g.) had m. p. 129° (Ohle and Thiel, *Ber.*, 1933, **66**, 528, give m. p. 129—130°).

This monotosyl compound (0·74 g.) was dissolved in dry pyridine (2·5 c.c.) and allowed to react with *p*-toluenesulphonyl chloride (0·56 g.) for 6 hours at room temperature and for 2 days at 30°. The reaction mixture was treated with a few drops of water, and after 15 minutes was diluted with chloroform (20 c.c.). The chloroform solution was washed with dilute sulphuric acid, sodium bicarbonate solution, water, and then dried over anhydrous magnesium sulphate. After filtration, followed by removal of chloroform and crystallisation from ethyl alcohol, the 2 : 6-ditosyl 3 : 4-monoacetone α-methylgalactoside had m. p. 148°; $[\alpha]_D^{18°}$ + 117° in pyridine (*c*, 0·3) (yield almost quantitative). This material gave no depression of the m. p. when mixed with that prepared as in (a).

A. *Preparation of* 2-*Methyl* 3 : 6-*Anhydro*-α-*methylgalactopyranoside* (XI).—6-*Tosyl* 3 : 4-*monoacetone* 2-*methyl* α-*methylgalactopyranoside* (IX).—A solution of 6-tosyl 3 : 4-monoacetone α-methylgalactoside (0·5 g.) in methyl iodide (5 c.c.) was refluxed for 6 hours in the presence of silver oxide (3 g.), the latter being added in small quantities during the first 3 hours. The excess of the methyl iodide was distilled, and the residue extracted with hot acetone. Removal of the solvent gave an almost quantitative yield of crystalline 6-*tosyl* 3 : 4-*monoacetone* 2-*methyl* α-*methylgalactoside*, m. p. 88°; $[\alpha]_D^{22°}$ + 99° in pyridine (*c*, 0·5) (after recrystallisation from ethyl alcohol–light petroleum). The compound is readily soluble in methyl and ethyl alcohol, ether, and pyridine, but insoluble in water and light petroleum (Found : C, 53·65; H, 6·45; OMe, 15·85; S, 7·7. $C_{18}H_{26}O_8S$ requires C, 53·7; H, 6·5; OMe, 15·4; S, 7·95%).

6-*Tosyl* 2-*methyl* α-*methylgalactopyranoside* (X). When a solution of 6-tosyl 3 : 4-monoacetone 2-methyl α-methylgalactoside (0·59 g.) in 1% methyl-alcoholic hydrogen chloride (100 c.c.) was refluxed for 4 hours, it showed $[\alpha]_D$ + 98° (initial value); + 64° (after 1 hour); + 41° (2 hours); + 27° (3 hours); + 27° (4 hours). Neutralisation of the solution with silver oxide, followed by filtration and removal of the solvent under reduced pressure, gave a colourless syrup (0·48 g.) which had $[\alpha]_D^{23°}$ + 27° in ethyl alcohol (*c*, 0·8). The syrupy 6-*tosyl* 2-*methyl methylgalactoside* did not reduce boiling Fehling's solution (Found : OMe, 16·6. $C_{15}H_{22}O_8S$ requires OMe, 17·1%).

2-*Methyl* 3 : 6-*anhydro*-α-*methylgalactoside* (XI). A solution of the syrupy 6-tosyl 2-methyl α-methylgalactoside (0·47 g.) in ethyl alcohol (10 c.c.) was heated with N-sodium hydroxide (2 c.c.) for 1 hour at 60°. The mixture was neutralised to phenolphthalein by a current of carbon dioxide and evaporated to dryness under reduced pressure. Extraction of the residue with ethyl alcohol gave syrupy 2-methyl 3 : 6-anhydromethylgalactoside, which distilled as a colourless liquid, b. p. (bath temp.) 165°/0·03 mm., $n_D^{16°}$ 1·4710, $[\alpha]_D^{21°}$ + 63° in ethyl alcohol (*c*, 0·3) (Found : OMe, 32·0%). After 3 days the distillate crystallised spontaneously, and when purified by recrystallisation from ethyl alcohol–light petroleum the 2-*methyl* 3 : 6-*anhydro*-α-*methylgalactopyranoside* had m. p. 102°; $[\alpha]_D^{18°}$ + 88° in water (*c*, 0·4) (Found : C, 50·0; H, 7·2; OMe, 32·0. $C_8H_{14}O_5$ requires C, 50·6; H, 7·4; OMe, 32·6%).

One treatment of this galactoside (0·03 g.) with silver oxide (0·5 g.) and methyl iodide (2 c.c.) under reflux for 6 hours yielded 2 : 4-dimethyl 3 : 6-anhydro-α-methylgalactoside (isolated by means of acetone). Exposure of the syrupy product to the action of dry hydrogen chloride for 30 seconds effected rapid isomerisation, and the 2 : 4-dimethyl 3 : 6-anhydro-β-methylgalactopyranoside readily crystallised (Haworth, Jackson, and Smith, J., 1940, 620). The product was dissolved in ether (2 c.c.), neutralised with silver oxide, filtered, and concentrated to dryness. One crystallisation from 1—2 parts of water gave the β-galactoside, m. p. and mixed m. p. 82°.

B. *Synthesis of* 4-*Methyl* 3 : 6-*Anhydro*-α-*methylgalactopyranoside* (VII).—Before attempting to synthesise this compound, the conditions were ascertained which would bring about the removal of the tosyl group from C_2 as well as the formation of the 3 : 6-anhydro-ring. It was found that when a solution of 2 : 6-ditosyl α-methylgalactopyranoside (0·1 g.) in ethyl alcohol (10 c.c.) and aqueous sodium hydroxide (5 c.c., 12%) was heated at 60° until a drop of the solution gave no turbidity on dilution with water, there was produced 3 : 6-anhydro-α-methylgalactoside. The solution was neutralised with carbon dioxide, and evaporated to dryness under diminished pressure. Extraction of the residue with ethyl alcohol gave 3 : 6-anhydro-α-methylgalactopyranoside, m. p. and mixed m. p. 139° (after recrystallisation from ethyl acetate).

2-*Tosyl* 3 : 6-*anhydro*-α-*methylgalactoside* (V). A solution of 2 : 6-ditosyl α-methylgalactoside (1 g.) in a mixture of ethyl alcohol (10 c.c.) and N-sodium hydroxide (3 c.c.) was heated for 1 hour at 60°. Carbon dioxide was passed through

the solution until this became neutral to phenolphthalein and it was then evaporated to dryness under diminished pressure. Extraction of the crystalline residue with acetone gave crystalline *2-tosyl* 3 : 6-*anhydro-α-methylgalactopyranoside* (0·8 g.), m. p. 138°; $[\alpha]_D^{18°}$ + 56° in chloroform (*c*, 0·8) (after recrystallisation from ethyl alcohol) (Found: C, 50·7; H, 5·45; OMe, 9·5; S, 9·5. $C_{14}H_{18}O_7S$ requires C, 50·9; H, 5·5; OMe, 9·4; S, 9·7%). The substance is slightly soluble in ethyl and methyl alcohol, readily soluble in pyridine and chloroform. In another experiment 3 g. of the initial substance afforded 2·45 g. of the product.

2-Tosyl 4-methyl 3 : 6-*anhydro-α-methylgalactopyranoside* (VI). A solution of (V) (1 g.) in methyl iodide (5 c.c.) was refluxed for 6 hours with silver oxide (3 g.), the latter being added in small portions during the first 3 hours. After isolation by means of acetone, the product crystallised spontaneously, and when recrystallised from ethyl alcohol, in which it is sparingly soluble in the cold, the *2-tosyl 4-methyl* 3 : 6-*anhydro-α-methylgalactopyranoside* had m. p. 126°; $[\alpha]_D^{23°}$ + 88° in chloroform (*c*, 0·4). The compound is readily soluble in pyridine and chloroform (Found: C, 53·1; H, 5·9; OMe, 18·7; S, 9·3. $C_{15}H_{20}O_7S$ requires C, 52·3; H, 5·8; OMe, 18·0; S, 9·3%).

4-Methyl 3 : 6-*anhydro-α-methylgalactopyranoside* (VII). A solution of the preceding compound (1·0 g.) in ethyl alcohol (10 c.c.) and aqueous sodium hydroxide (5 c.c., 12%) was heated for 30 hours at 60°, a drop of the reaction mixture then no longer giving a turbidity when added to water. The mixture was neutralised with carbon dioxide, and evaporated to dryness under reduced pressure. Extraction of the residue with acetone gave *4-methyl* 3 : 6-*anhydro-α-methylgalactoside* as a syrup, which distilled as a colourless oil (0·4 g.), b. p. (bath temp.) 110°/0·03 mm.; $n_D^{20°}$ 1·4795; $[\alpha]_D^{19°}$ + 64° in water (*c*, 0·4). After two days the distillate crystallised, and recrystallisation from ethyl alcohol gave (VII); m. p. 55°; $[\alpha]_D^{19°}$ + 81° in methyl alcohol (*c*, 1·1); $[\alpha]_D^{16°}$ + 75° in water (*c*, 0·6) (Found: C, 50·6; H, 7·0; OMe, 32·4. $C_8H_{14}O_5$ requires C, 50·6; H, 7·4; OMe, 32·6%).

Methylation of 4-Methyl 3 : 6-*Anhydro-α-methylgalactopyranoside.*—Two treatments of the crystalline galactoside (0·1 g.) with silver oxide (1 g.) and methyl iodide (3 c.c.) gave 2 : 4-dimethyl 3 : 6-anhydro-α-methylgalactoside. After each methylation the product was isolated by means of acetone. Removal of the solvent gave a colourless, mobile liquid (0·11 g.); b. p. (bath temp.) 100°/0·02 mm.; $n_D^{18°}$ 1·4660; $[\alpha]_D^{18°}$ + 75° in water (*c*, 0·5) (Found: OMe, 45·7. Calc. for $C_9H_{16}O_5$: OMe, 45·6%). Treatment of this syrupy compound (20 mg.) with dry hydrogen chloride for about 30 seconds effected rapid isomerisation (Haworth, Jackson, and Smith, *loc. cit.*) and the β-form readily crystallised. The crystalline mass was dissolved in dry ether (5 c.c.), and any mineral acid neutralised with a little silver oxide. The solution was filtered and evaporated to dryness. One crystallisation of the product from 1—2 parts of water gave pure 2 : 4-dimethyl 3 : 6-anhydro-β-methylgalactopyranoside, m. p. and mixed m. p. 83°; $[\alpha]_D^{15°}$ − 78° in water (*c*, 0·5).

When conditions permit a more detailed examination of the 2-monomethyl and the 4-monomethyl 3 : 6-anhydro-α-methylgalactoside will be made.

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59. *The Isomerisation of some Aromatic Ketones with Aluminium Chloride.*

By G. BADDELEY.

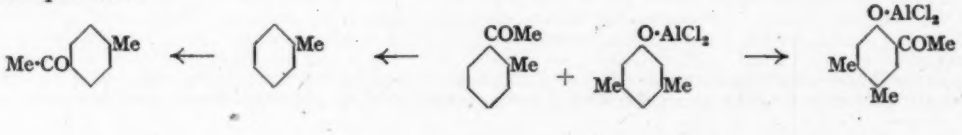
These isomerisations are shown to be of two types; the mechanism of each has been determined, and the factors controlling the relative amounts of isomerisation of each type are indicated. The migrations of alkyl groups in benzene homologues, phenols, aryl ketones, and hydroxyaryl ketones are related to one another and to the Jacobsen reaction.

IT was suggested (J., 1943, 273) that elucidation of the isomerisation of 2-hydroxyaryl alkyl ketones by aluminium chloride required a study of the action of this reagent on alkylphenols and on aryl alkyl ketones. The former has already been contributed (J., 1943, 527) and the latter is now supplied.

The following isomerisations have been realised by the action of aluminium chloride:

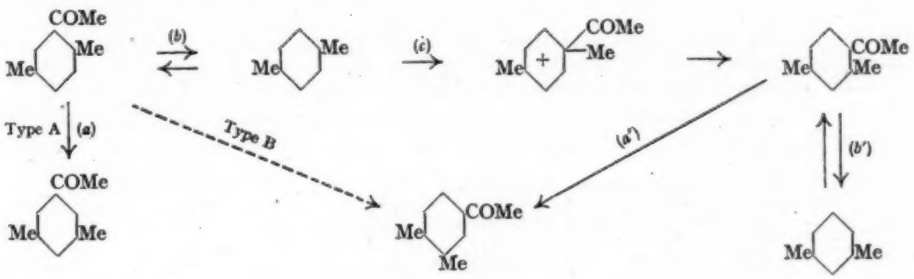
	Products of isomerisation.	
Initial ketone.	Type A.	Type B.
2-C_6H_4Me·COMe	None	4-C_6H_4Me·COMe (85%)
2-C_6H_4Me·COEt	None	4-C_6H_4Me·COEt (83%)
2 : 5-$C_6H_3Me_2$·COMe	3 : 5-$C_6H_3Me_2$·COMe (77%)	3 : 4-$C_6H_3Me_2$·COMe (8%)
2-C_6H_4Et·COMe	3-C_6H_4Et·COMe (70%)	4-C_6H_4Et·COMe (small)
2 : 5-$C_6H_3Et_2$·COMe	3 : 5-$C_6H_3Et_2$·COMe (83%)	None
2 : 4-$C_6H_3Me_2$·COMe	3 : 4-$C_6H_3Me_2$·COMe (80%)	None
2 : 4 : 6-$C_6H_2Me_3$·COMe	3 : 4 : 5-$C_6H_2Me_3$·COMe (87%)	None
2 : 5-$C_6H_3Me_2$·COPh	3 : 5-$C_6H_3Me_2$·COPh (90%)	None
5 : 8-Dimethyl-α-tetralone	5 : 7-Dimethyl-α-tetralone (90%)	None
4 : 7-Dimethyl-α-hydrindone	No change	

Acetophenone is converted into dypnone, 2CPhMe:O ⟶ CPhMe:CH·COPh, when heated with less than 1 mol. of aluminium chloride (Calloway and Green, *J. Amer. Chem. Soc.*, 1937, **59**, 809). This type of reaction can be suppressed by ensuring that the ketone is entirely engaged by the chloride, and unchanged acetophenone is recovered after treatment with excess of reagent at 150—180°. Under such conditions, at least 2 mols. of aluminium chloride being used, 2-methyl-aceto- and -propio-phenones are converted into the 4-methyl isomerides in excellent yield. If, however, the reaction be carried out in presence of *m*-5-xylenol, the yield of 4-methylacetophenone is halved owing to formation of 2-hydroxy-4 : 5-dimethylacetophenone, and it is thus reasonable to assume that the formation of an acylating agent is also responsible for the production of 4-methylacetophenone:



If an appreciable concentration of toluene were produced in the isomerisation of the *o*-tolyl ketone, the redistribution of alkyl groups, which this hydrocarbon is known to undergo in the presence of aluminium chloride, would be expected to lead to the presence of acetophenone and dimethylacetophenone among the products of the reaction. These, however, are not found.

Again, the subsidiary formation of 3 : 4-dimethylacetophenone from 2 : 5-dimethylacetophenone (the 3 : 5-isomeride being the main product) is completely suppressed by the presence of *m*-5-xylenol, and the reaction is perhaps best formulated as follows :



The acetylation of *p*-xylene to 2 : 4-dimethylacetophenone is assumed to involve a Wagner–Meerwein rearrangement and is related to the formation of *p-tert.*-butylacetophonone from *p*-di-*tert.*-butylbenzene by the action of acetyl chloride and aluminium chloride (Hennion and McLeese, *J. Amer. Chem. Soc.*, 1942, **64**, 2421). The Fries rearrangement of 4 : 6-dimethyl-2-ethylphenyl acetate to 2-hydroxy-3 : 5-dimethyl-4-ethylacetophenone (Auwers, *Annalen*, 1928, **460**, 240) is similarly explained. The conversion of 2 : 5- into 2 : 4-dimethylacetophenone is described later; it does not involve the isomerisation of *p*- to *m*-xylene, since this reaction is reversible and the conversion of 2 : 4- into 3 : 5-dimethylacetophenone has not been observed.

These isomerisations are of two types : (A) Those resembling the isomerisation of *o*-hydroxyaryl ketones, the mobile alkyl group moving intramolecularly into the neighbouring position, and (B) those involving the migration (possibly intramolecular) of the carbonyl group. The relative amounts of isomerisation of these two types are determined by the following factors which are indicated in the above diagram : (*a*) The mobility of the alkyl group, (*b*) the ease of fission of the acetyl group from the nucleus, (*c*) the probability of the resynthesis producing a different ketone.

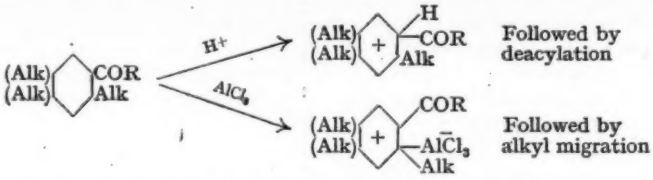
Another alkyl group in the 4-position to the one undergoing migration was found to facilitate the type A isomerisation of *o*-hydroxyketones (J., 1943, 273); this effect, enhanced factor (*a*), explains the conversion of 2 : 5- into mainly 3 : 5-dimethylacetophenone, whereas 2-methyl- gives only 4-methyl-acetophenone. Again, the greater mobility of the ethyl group is now demonstrated by the conversion of 2- into 3-ethylacetophenone, and of 2 : 5- into 3 : 5-diethylacetophenone without the formation of the 3 : 4-isomeride.

Benzophenone is stable up to 200° with any amount of aluminium chloride, and the oxonium complex has been partly distilled under reduced pressure. In consequence of this stability, the isomerisation of 2 : 5- into 3 : 5-dimethylbenzophenone is shown to require more than 1 mol. of reagent. Again, the velocity constants for the isomerisation of 6-hydroxy-2 : 4- into 6-hydroxy-3 : 4-dimethylacetophenone in the presence of added 2 : 4- or 3 : 4-dimethylacetophenone show that both these ketones use only 1 mol. of reagent. Consequently, after oxonium complex formation, further additions of reagent act catalytically in isomerising aromatic ketones.

p-Hydroxyacetophenones undergo only isomerisations of type B, and the complete isomerisation of 4-hydroxy-2-methyl- into 2-hydroxy-4-methyl-acetophenone (Rosenmund and Schnurr, *Annalen*, 1928, **460**, 56) was achieved with only a fraction over 1 mol. of aluminium chloride. Now the isomerisation of 2 : 5- into 2 : 4-dimethylacetophenone has been realised by heating the former with a mixture of 1 mol. of 6-hydroxy-3 : 4-dimethylacetophenone and 3 mols. (one being engaged by the ketone and two by the hydroxy-ketone) of aluminium chloride; this is an isomerisation of type B occurring under conditions where there is no reagent capable of producing an isomerisation of type A.

The acyl group which is displaced or the alkyl group which migrates is in the *o*-position to a bulky group, and it is suggested that it is this steric factor which determines the various changes now under discussion. Further, 5 : 8-dimethyl-α-tetralone undergoes isomerisation by aluminium chloride to its 5 : 7-isomeride and hydrolytic fission of the ketonic ring by phosphoric acid, whereas 4 : 7-dimethyl-α-hydrindone, with a *rigid* and *planar* structure, remains unchanged. This difference suggests that the isomerisation of an aromatic ketone requires the propulsion of the carbonyl group out of the plane of the aromatic nucleus. Since the migration of an alkyl group away from a carbonyl group requires the attachment of a second molecule of aluminium chloride, the first forming an oxonium complex, it was suggested (*Nature*, 1939, **144**, 444) that the orientation of the carbonyl group out of the plane of the nucleus inhibited, at least in part, the mesomeric effect of the carbonyl group and thus augmented the susceptibility of the nucleus to electrophilic attack. Moreover, since mobility is only conferred on the carbonyl group and an *o*-alkyl group, it was further suggested that the collisions between these two encourage a polarisation of the nucleus which enhances the electron

availability at one or other of the positions to which these groups are attached (*loc. cit.*; *ibid.*, 1942, **150**, 178):

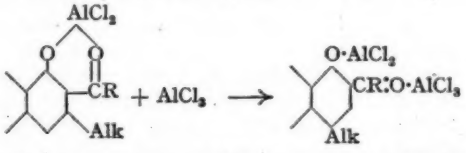


This readily explains why the isomerisation of type A is accelerated by an alkyl group in the *p*-position to the one undergoing migration.

The relative rates of deacylation and alkyl-group migration are revealed, since the former may not lead to an isomerisation of type B, by the addition of *m*-5-xylenol, which is acylated to a 2-hydroxy-4 : 5-dimethylaryl ketone. The results are summarised in the following table:

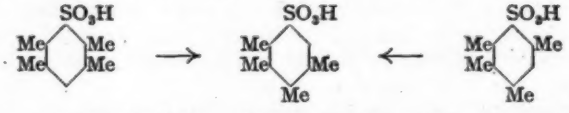
Initial ketone.	Recovered ketone.	Hydroxy-ketone.
2-C_6H_4Me·COMe	4-C_6H_4Me·COMe (42%)	40%
2 : 4-$C_6H_3Me_2$·COMe	3 : 4-$C_6H_3Me_2$·COMe (50%)	32%
2 : 5-$C_6H_3Me_2$·COMe	3 : 5-$C_6H_3Me_2$·COMe (75%)	8%
3 : 4-$C_6H_3Me_2$·COMe	3 : 4-$C_6H_3Me_2$·COMe (80%)	trace

All the isomerisations of *o*-hydroxyaryl ketones which have been examined are of type A. This does not signify that fission does not occur, but rather that it is completely reversible. This reversibility is due to the directing effect of the phenolic oxygen atom and the complete reversibility of the production of any *p*-hydroxyaryl ketone, coupled with (i) fission producing a symmetrical phenol homologue, or (ii) one *o*-position of the phenol nucleus being occupied by an alkyl group. Since both the hydroxyl and the carbonyl group usually use 1 mol. of aluminium chloride, a steric effect must account for the loss of only 1 mol. to a 6-hydroxy-2-alkylaryl ketone, and the isomerisation can, perhaps, be best formulated:



The isomerisation of aryl and hydroxyaryl ketones also occurs with aluminium bromide as reagent.

Homologues of benzonitrile are not isomerised by aluminium chloride; the nitrile group is linear and cannot collide with an *o*-alkyl group. Consequently, (i) the mesomeric effect of the nitrile group is not reduced by displacement of the nitrogen atom out of the plane of the nucleus, and (ii) the nitrile group as a whole and the neighbouring alkyl group as a whole are not displaced from the plane of the nucleus. On the other hand, homologues of benzenesulphonic acid can behave similarly to those of acetophenone: (i) an *o*-alkyl group facilitates the hydrolysis of the sulphonic acid, and (ii) an *o*-alkyl group can be displaced (by acid reagents); this is the Jacobsen reaction (*Ber.*, 1886, **19**, 1209), exemplified by:



Further, hexamethylbenzene was obtained from durene by the action of concentrated sulphuric acid; this disproportionation resembles those obtained by the action of aluminium chloride on benzene homologues.

The displacement by aluminium chloride of an alkyl group in an aromatic nucleus has been shown to require the juxtaposition of a bulky group. An acyl group (in an oxonium condition) provides this condition in the aromatic ketones and hydroxy-ketones, whereas the preliminary attachment of aluminium chloride is usually required with the homologues of benzene and phenol. The connection between a high electron availability of the nucleus and a ready intermolecular migration (J., 1943, 529) is now further illustrated by the aromatic ketones; no internuclear migration of alkyl groups occurs.

The rearrangement of the xylenes has been obtained (Norris and Vaala, *J. Amer. Chem. Soc.*, 1939, **61**, 2131) with only little disproportionation or decomposition; in consequence, the intramolecular migration of methyl groups by aluminium chloride has been obtained with aromatic hydrocarbons (at 50°), phenols (at 100—135°), and aromatic ketones and hydroxy-ketones (at 130—190°). The alkylation of phenols, acetophenone, and benzonitrile has been studied (later communications) and further illustrates that phenols and aromatic ketones have been isomerised without dealkylation and subsequent realkylation.

Experimental.

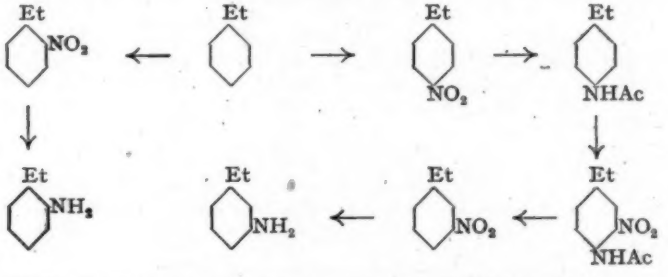
The ketones were mixed with aluminium chloride (2 mols.) and heated at 170° for 1·5 hours. The product was poured on ice, extracted with ether, dried over potassium carbonate, and distilled under reduced pressure. The products were identified by the m. p. and mixed m. p. of the semicarbazones.

2-Methylacetophenone (10 g.) and aluminium chloride (20 g.) produced 4-methylacetophenone (8·5 g.), and 2-methylpropiophenone (18 g.) and reagent (36 g.) produced 4-methylpropiophenone (15 g.). 2 : 5-Dimethylacetophenone (90 g.) and the chloride (170 g.) gave a product (75 g.), b. p. 118—122°/24 mm. After repeated fractional distillation, the first and the last fraction were identified as 3 : 5-dimethyl- and 3 : 4-dimethyl-acetophenone, respectively. The semicarbazone (m. p. 195—205°) of the mixture of ketones indicated 90% and 10% respectively of the two ketones.

2-Ethylacetophenone (9 g.) and reagent (18 g.) produced 3-ethylacetophenone (6·5 g.), contaminated with a little of the 4-isomeride. 2 : 5-Diethylacetophenone (53 g.) and aluminium chloride (84 g.) gave 3 : 5-diethylacetophenone (44 g.). Reduction by the Clemmensen method gave 1 : 3 : 5-triethylbenzene, identified by its trinitro- and tribromo-derivatives (m. p. 109° and 105—106°, respectively; Gattermann, Fritz, and Beck, *Ber.*, 1899, **32**, 1124). From alcohol, the former crystallised in long, colourless needles, m. p. 112° (Found : N, 14·1. Calc. for $C_{12}H_{15}O_6N_3$: N, 14·1%), and the latter had m. p. 105° (Found : Br, 60·0. Calc. for $C_{12}H_{15}Br_3$: Br, 60·2%). 2 : 4-Dimethylacetophenone (10 g.) and the chloride (20 g.) gave 3 : 4-dimethylacetophenone (8 g.). Mesityl methyl ketone (8 g.) and the chloride (16 g.) gave 3 : 4 : 5-trimethylacetophenone (7 g.), oxidised by alkaline hypochlorite to 3 : 4 : 5-trimethylbenzoic acid. 2 : 5-Dimethylbenzophenone (14 g.) and reagent (20 g.) were heated together at 190° for 2 hours. The recovered ketone (13 g.) crystallised from light petroleum (b. p. 40—60°) in stout, colourless needles, m. p. 70°, which did not depress the m. p. (70°) of 3 : 5-dimethylbenzophenone.

5 : 8-Dimethyl-α-tetralone (2 g.) and reagent (5 g.) gave 5 : 7-dimethyl-α-tetralone (1·8 g.), whereas 4 : 7-dimethyl-α-hydrindone and those ketones with no alkyl group in the *o*-position to the carbonyl group did not isomerise and were recovered to the extent of *ca.* 85%.

Preparation of the Ketones.—2- and 3-Methyl- and 2- and 3-ethyl-acetophenone and 2- and 3-methylpropiophenone were obtained in excellent yield from the appropriate homologue of benzonitrile (prepared from the corresponding amine) and the appropriate Grignard reagent. 2-Ethyl- and 3-ethyl-aniline were obtained in the following manner :



The mixture of mononitroethylbenzenes was separated by repeated fractional distillation. 3 : 5-Dimethylacetophenone was obtained in poor yield from acetonitrile and 3 : 5-dimethylphenylmagnesium bromide. 5-Bromo-*m*-xylene was obtained from *m*-4-xylidine.

5 : 8-*Dimethyl-α-tetralone.* β-(2 : 5-Dimethylbenzoyl)propionic acid (m. p. 62°, Muhr, *Ber.*, 1895, **28**, 3215) was obtained by shaking together molecular proportions of *p*-xylene, succinic anhydride, and aluminium chloride in carbon disulphide for 3 days. It crystallised from water in needles, m. p. 80° (Claus, *Ber.*, 1887, **20**, 1374, gives m. p. 84°). This ketone was reduced by Clemmensen's method to γ-(2 : 5-dimethylphenyl)butyric acid, b. p. 197°/30 mm., m. p. 70°. The acid chloride, obtained by addition of thionyl chloride, was dissolved in carbon disulphide, and aluminium chloride (1·2 mols.) added. Hydrogen chloride was readily evolved, and the tetralone was obtained as an oil, b. p. 164°/20 mm.

5 : 7-*Dimethyl-α-tetralone.* This was obtained from *m*-xylene in the manner described for the 5 : 8-isomeride. β-(2 : 4-Dimethylbenzoyl)propionic acid crystallised from dilute acetic acid in plates, m. p. 113° (Claus, *loc. cit.*, gives m. p. 108°), and γ-(2 : 4-dimethylphenyl)butyric acid in plates, m. p. 79° (Claus, *J. pr. Chem.*, 1892, **46**, 476, gives m. p. 70°).

4 : 7-*Dimethyl-α-hydrindone.* *p*-Xylene (10·6 g.), β-chloropropionic acid (10·9 g.), and aluminium chloride (15 g.) were heated together at 100° until evolution of hydrogen chloride ceased. Addition of water gave a solid aluminium salt, which was separated and decomposed with dilute sulphuric acid. β-(2 : 5-Dimethylphenyl)propionic acid was extracted with ether, dried over calcium chloride, and distilled; b. p. 183°/25 mm. (8 g.). The acid chloride was prepared by addition of thionyl chloride (6 g.), excess of which was removed at 100°/25 mm. Hydrogen chloride was readily evolved when aluminium chloride (7·5 g.) was added to a solution of the acid chloride in carbon disulphide (30 c.c.). The ketone was isolated in the usual manner; b. p. 150°/20 mm. (5 g.), and m. p. 77° after crystallisation from light petroleum (b. p. 60—80°) (Moureu, *Bull. Soc. chim.*, 1893, **9**, 572, gives m. p. 77—78°). All the other ketones were prepared by Friedel–Crafts reactions between the appropriate benzene homologue and acid chloride. 3 : 5-Dimethylbenzoyl chloride, required in the preparation of 3 : 5-*dimethylbenzophenone*, m. p. 70° (Found : C, 85·6; H, 6·9. $C_{15}H_{14}O$ requires C, 85·7; H, 6·7%), was obtained by oxidation of mesitylene by dilute nitric acid and subsequent addition of thionyl chloride to the resulting 3 : 5-dimethylbenzoic acid. *o*-Diethylbenzene, required in the preparation of 3 : 4-diethylacetophenone, was obtained by Clemmensen reduction of 2-ethylacetophenone.

Action of Aluminium Chloride in the Presence of m-5-*Xylenol.*—2 : 5-Dimethylacetophenone (10·8 g.), the xylenol (9·0 g.), and aluminium chloride (39 g.) were heated together at 160° for 4 hours. The mixture was then poured on ice, the ethereal extract washed with dilute sodium hydroxide solution, and dried over potassium carbonate. Pure 3 : 5-dimethylacetophenone (8·1 g.) was obtained on distillation. The alkaline washings were acidified with dilute sulphuric acid, and the ethereal extract dried over calcium chloride. The residue (*ca.* 1 g.), after removal of the xylenol by distillation, crystallised from light petroleum (b. p. 60—80°) in plates, m. p. 74°, which did not depress the m. p. of 2-hydroxy-4 : 5-dimethylacetophenone. 2 : 4-Dimethylacetophenone (14 g.), the xylenol (12 g.), and aluminium chloride (42 g.) were treated as in the above experiment; 3 : 4-dimethylacetophenone (7 g.) and 2-hydroxy-4 : 5-dimethylacetophenone (6 g.) were obtained. 3 : 4-Dimethylacetophenone was treated exactly as in the previous experiment; unchanged ketone (11 g.) and traces of hydroxy-ketone were obtained. 2-Methylacetophenone (6 g.), the xylenol (7 g.), and aluminium chloride (40 g.) were treated as in the above experiments; 4-methylacetophenone (2·5 g.) and hydroxy-ketone (3·0 g.) were obtained.

Hydrolysis with Syrupy Phosphoric Acid.—All the *o*-alkylaryl ketones and 5 : 8-dimethyl-α-tetralone, but *not* 4 : 7-dimethyl-α-hydrindone, were readily hydrolysed by syrupy phosphoric acid at 180°, the carbonyl group becoming detached from the aromatic nucleus.

Isomerisations with Aluminium Bromide.—6-Hydroxy-2 : 4-dimethyl- and 2 : 4-dimethyl-acetophenone produced

2-hydroxy-4 : 5-dimethyl- and 3 : 4-dimethyl-acetophenone, respectively, when heated with aluminium bromide (3 mols.) at 150° for 3 hours. Quantitative measurements were not made, but the isomerisations appeared to occur at least as readily as when aluminium chloride was used.

Aluminium Chloride and Homologues of Benzonitrile.—2 : 5-Dimethylbenzonitrile (10 g.) and the reagent (22 g.) were heated together at 220—230° for an hour. Some darkening occurred, but unchanged nitrile (9 g.) was recovered. The corresponding acid amide was used for identification. Similarly, *o*-toluonitrile under the above conditions showed no isomerisation.

Isomerisation of 2 : 5-Dimethyl- into 2 : 4-Dimethyl-acetophenone.—2 : 5-Dimethylacetophenone (7·4 g.), *o*-4-xylenyl acetate (3·6 g.), and aluminium chloride (12·7 g.) were heated together at 160° for 5 hours. The recovered alkali-insoluble fraction (6 g.) was a mixture of 2 : 5-dimethyl- (*ca.* 70%) and 2 : 4-dimethyl-acetophenone. The semicarbazone of the former was less soluble in ethyl alcohol than was that of the latter.

2 : 4-Dinitrophenylhydrazones.

Ketone.	B. p.	M. p. of deriv.	N, %. Found.	Calc.
4-C_6H_4Et·COMe	114°	203°	17·1	17·1
2 : 5-$C_6H_3Et_2$·COMe	125	105	15·8	15·7
3 : 5- ,,	130	185	15·8	15·7
4-$C_6H_4Pr^a$·COEt	131	147	15·9	15·7
2 : 5-$C_6H_3Pr^a_2$·COMe	145	75	14·6	14·6

Semicarbazones.

Ketone.	B. p./20 mm.	M. p. of deriv.	N, %. Found.	Calc.
2-C_6H_4Me·COMe	94°	212°	22·2	22·0
3- ,,	102	205	22·1	,,
4- ,,	102	208	22·1	,,
2-C_6H_4Me·COEt	108	173	(a)	
3- ,,	115	178	(b)	
4- ,,	115	187	(c)	
2-C_6H_4Et·COMe	106	182	20·5	20·5
3- ,,	114	175	20·5	,,
4- ,,	114	191	20·6	,,
2 : 4-$C_6H_3Me_2$·COMe	110	202	20·4	20·5
2 : 5- ,,	110	168	(d)	
3 : 4- ,,	122	235	(e)	
3 : 5- ,,	118	219	20·6	
3 : 4-$C_6H_3Et_2$·COMe	133	180	17·9	18·0
3 : 5- ,,	130	149	18·2	,,
3 : 4 : 5-$C_6H_2Me_3$·COMe	138	217	19·3	19·2
α-Tetralone	127	226	20·7	20·7
5 : 7-Me_2-α-tetralone	170	245	18·1	18·2
5 : 8- ,,	164	222	18·2	,,
$C_6H_4Pr^a$·COEt	131	143	18·0	18·0

(a) Blaise, *Compt. rend.*, 1901, **133**, 1218. (b) Wallach and Rentschler, *Annalen*, 1908, **360**, 62. (c) Auwers, *Annalen*, 1915, **408**, 243; *Ber.*, 1916, **49**, 2400. (d) Auwers, *loc. cit.* (e) Auwers and Kochritz, *Annalen*, 1907, **352**, 306.

The author is indebted to Professor J. Kenner, F.R.S., for his interest in the work described in this paper.

COLLEGE OF TECHNOLOGY, MANCHESTER. [*Received, June 8th*, 1942.]

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60. *The Mechanism of the Reaction of (−)Phenylalkylcarbinols with Hydrogen Bromide.*

By C. L. ARCUS.

Levene and Rothen (*J. Biol. Chem.*, 1939, **127**, 237), having determined the rotations of the bromides produced by the action of hydrogen bromide on (−)phenyl-methyl-, -ethyl-, and -*n*-propyl-carbinols at temperatures from −80° to +20°, expressed the view that the three mechanisms of substitution known as S_Ni, S_N2, and S_Ni do not suffice to explain these curves. An explanation is now put forward in which the participation of each mechanism in the total reaction is represented by a distribution curve about a maximum at a certain temperature, and it is found that the algebraic sum of the optical results of the three mechanisms reproduces the experimental curves.

The "domain" of each mechanism, represented by the area between its distribution curve and the temperature axis, has been calculated for the three reactions.

LEVENE and ROTHEN (*loc. cit.*) have determined the sign and magnitude of the rotation of the α-phenyl-ethyl, -*n*-propyl, and -*n*-butyl bromides produced by the action of hydrogen bromide, in the absence of solvent, on (−)phenyl-methyl-, -ethyl-, and -*n*-propyl-carbinols, at temperatures from −80° to +20°. The percentage optical purity of these bromides varies with temperature of reaction according to Fig. 1 for α-phenylethyl-bromide (the propyl analogue gives a very similar curve) and to Fig. 2 for the *n*-butyl compound. Values for the first two compounds approach large negative limits at −80°; with increasing temperature they fall to zero, and then rise to a positive maximum, from which they slowly fall. The (−)α-phenyl-*n*-butyl bromide produced

at −80° appears to have reached a limiting negative optical purity; with increasing temperature a curve is followed similar in shape to those of the other two, but which is always negative.

The method employed was the same throughout. Dry hydrogen bromide was introduced very slowly into an evacuated flask containing the carbinol and maintained at the required temperature. The reaction was allowed to proceed substantially to completion. The excess hydrogen bromide was pumped off, and the bromide isolated and distilled. Hence, for a single carbinol the only variable is the reaction temperature, and for different carbinols reacting at the same temperature the only variable is the structure of the carbinol.

Three mechanisms of substitution applicable to these reactions have been described. A bimolecular mechanism leading to complete inversion has been proposed by Kenyon and Phillips (*Trans. Faraday Soc.*, 1930, **26**, 451) and investigated kinetically by Hughes, Ingold, and Masterman (J., 1937, 1196). For the reaction of an alcohol with hydrogen bromide this mechanism (S_N2) is

$$\rangle C{-}OH + HBr \longrightarrow Br^{\ominus}\ \rangle C{-}OH_2^{\oplus} \longrightarrow Br{-}C\langle + H_2O$$

A unimolecular mechanism was proposed by Hughes and Ingold (J., 1935, 244), in which the molecule undergoing substitution slowly ionises and the carbonium cation reacts with the entering group (S_N1):

$$\rangle C{-}OH_2^{\oplus} \longrightarrow \rangle C^{\oplus} + H_2O \quad \rangle C^{\oplus}\ Br^{\ominus} \longrightarrow \rangle C{-}Br$$

The carbonium cation is flat, but it is shielded from attack by the withdrawing group, whence the inverted configuration preponderates. Hughes, Ingold, and Scott (J., 1937, 1201) found the hydrolysis of α-phenylethyl chloride in water and aqueous acetone to be unimolecular and to result in 2—17·5% excess of the inverted configuration.

A third mechanism (S_Ni) for the replacement of hydroxyl by halogen has been described (Cowdrey, Hughes, Ingold, Masterman, and Scott, J., 1937, 1252; Hughes, Ingold, and Whitfield, *Nature*, 1941, **147**, 206); an addition compound is formed, and if sufficient electron release is available to the carbon atom undergoing substitution, a rearrangement leading to retention of configuration occurs.

$$\rangle C{-}O{-}H \cdots Br^{\delta\ominus}\ (\delta\oplus) \longrightarrow \rangle C{-}Br + H_2O$$

The addition compound is probably formed by hydrogen bonding. The valency angle of the hydrogen bond is 180°, but bending to 120° is known in the *o*-nitrophenols, and bending to a quasi-cyclic structure by electrostatic attraction between the bromine and the carbon atoms may occur as a preliminary to reaction.

From the literature, Hughes, Ingold, and Scott (*loc. cit.*) concluded that when phenylmethylcarbinol and α-phenylethyl bromide have the same sign of rotation they are of the same configuration; Levene and Rothen (*loc. cit.*) further concluded from an analysis of the rotatory dispersions of these compounds that phenyl-methyl-, -ethyl-, and -*n*-propyl-carbinols with the same sign of rotation have the same configuration, and similarly for the three bromides. Hence, for all three reactions retention of sign means retention of configuration.

At low temperatures, the retention of configuration in all three reactions, together with the formation of a white crystalline addition compound in the reaction with the ethyl carbinol, indicates that S_Ni predominates at such temperatures. This is superseded, with rising temperature, by replacement with inversion until −30°, −27·5°, and −17·5°, respectively, are reached for the three reactions, whereupon movement in the direction of retention occurs. Since this movement results in larger rotatory powers for α-phenyl-*n*-butyl bromide, Levene and Rothen concluded that the change is due, not to the commencement of racemisation, but to the coming into play of a second mechanism leading to retention and that " consequently the mechanisms (above) do not suffice . . . since, of the three only one proceeds with retention, whereas the present observations point to two mechanisms connected with retention."

It is now shown that, on certain assumptions, these three mechanisms suffice to explain the form of the rotation–temperature curves.

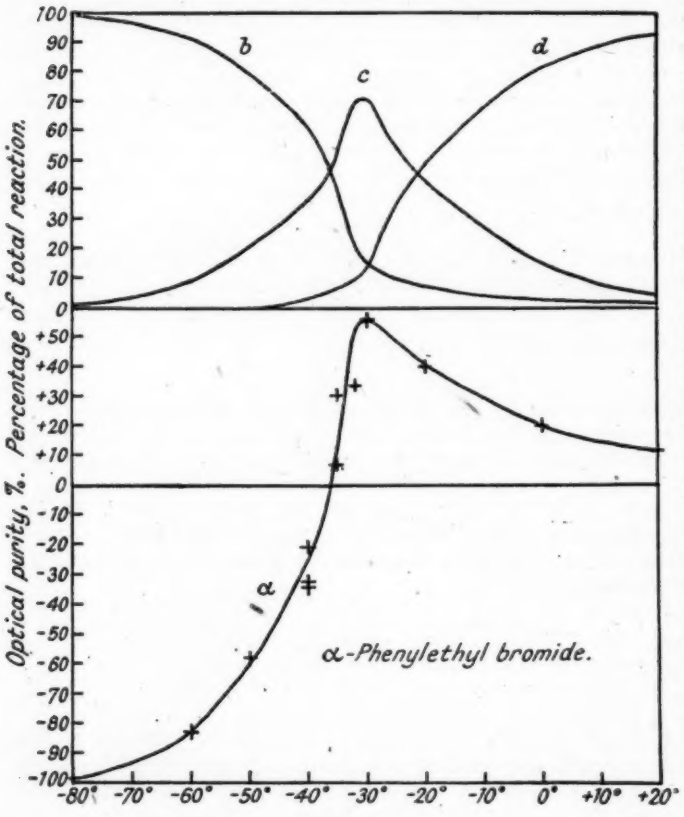
Mechanism S_N2 is considered to result in complete inversion; for S_N1, 10% inversion is estimated as an approximate value in the light of the results of Hughes, Ingold, and Scott (*loc. cit.*). The intramolecular mechanism S_Ni is considered to give complete retention. This is supported, for the phenylalkylcarbinols, by the almost complete retention observed during the (intramolecular) Hofmann and Curtius rearrangements

$$CHMePh{\cdot}CO{\cdot}NH_2 \longrightarrow CHMePh{\cdot}NH_2 \text{ and } CHMePh{\cdot}CO{\cdot}N_3 \longrightarrow CHMePh{\cdot}NH_2$$

in which the radical CHMePh· migrates (Arcus and Kenyon, J., 1939, 916; Kenyon and Young, J., 1941, 263). Then, considering the reaction of one carbinol with hydrogen bromide at one temperature, the phenylalkyl bromide is assumed to result from a combination of the three mechanisms, there being no experimental evidence for the existence of further mechanisms, applicable to the reaction conditions, of substitution of OH by Br. Since each mechanism has a characteristic optical result, it should be possible to determine, from the optical purity of the bromide, the percentage of the total reaction due to each mechanism. The bromide isolated was the product of the completed reaction, and hence these percentages refer to the reaction as a whole. The com-

position of the reaction medium changes as the reaction proceeds and the relative importance of the mechanisms may vary with time during the reaction; this aspect, however, is not discussed, and no assumptions are made about it. The percentages deduced are averages over the whole reaction period and their variations with changes in temperature and structure are described.

FIG. 1.



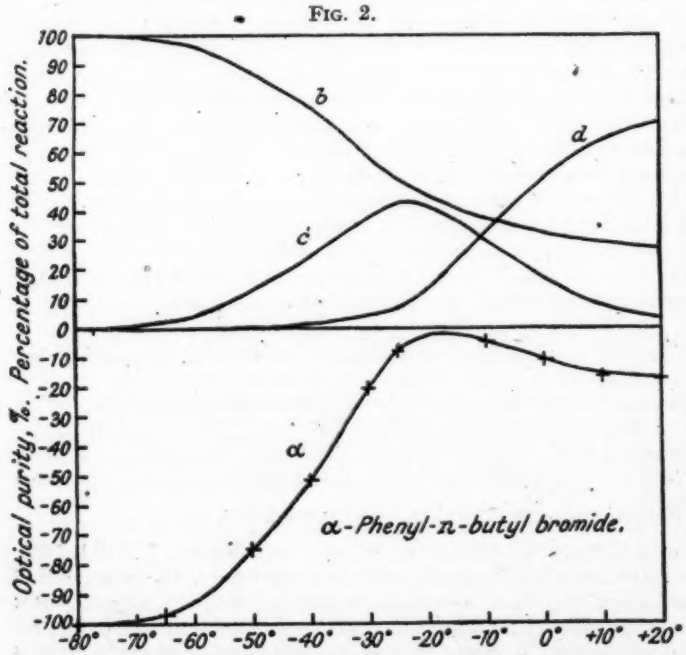
Finally, the assumption was made that the plot against temperature of the participation of any mechanism in the total reaction would, in general, be a distribution curve diminishing in a sigmoid curve on either side of a maximum at a certain temperature. (If one mechanism were to gain total control of the reaction above or below a certain temperature, then its maximum would be extended as a straight line over the range of total control.) Such a distribution curve represents the variation with temperature of the probability (the average probability over the whole reaction period) that a molecule of carbinol, when several mechanisms of reaction are possible to it, will react by one given mechanism.

The rotations of optically pure bromides were taken from the graphs of Levene and Rothen, small extrapolations being necessary for the methyl and the ethyl carbinol. The experimental rotations were then converted from $[M]_{5780}$ to percentage optical purity, and smooth curves were drawn through the resulting values; then, if b, c, and d are the percentages of the total reaction proceeding by mechanisms S_Ni, S_N2, and S_N1, respectively, and α is the percentage optical purity of the product, at a given temperature,

$$b + c + d = 100 \text{ and } \alpha = -(b - c - 0{\cdot}1d)$$

A third relation is supplied by the assumption that the curves b, c, and d are distribution curves which are always positive and have only one point of inflection between successive stationary points. It was then found by successive trial that if these conditions were to be satisfied the range of permissible variation is small. Representative values of b, c, and d have been calculated for 16 values of α for each bromide, and these values for α-phenyl-ethyl and -*n*-butyl bromide are plotted in Figs. 1 and 2, respectively.

FIG. 2.



Thus the three mechanisms S_Ni, S_N2, and S_N1, leading respectively to retention, inversion, and to much racemisation with some inversion (estimated at 10%), when their participations in the total reaction vary according to the distribution curves b, c, and d, yield bromides whose optical purity varies with temperature according to the experimental curves.

S_Ni approaches or attains a maximum at −80°, indicating that formation of the addition complex is favoured at low temperatures. S_N1 tends towards a maximum at above +20°, showing ionic fission of $R{\cdot}OH_2^{\oplus}$ to be predominant at higher temperatures. S_N2 is at a maximum at intermediate temperatures, approximately −25° to −30°, which are presumably low enough

to check ionisation and high enough to diminish the addition of hydrogen bromide which yields the complex of S_Ni.

In view of the assumptions made, the distribution curves are not numerically exact, but the following relationships may be noted. The "domain" of each mechanism between the temperatures considered, −80° to +20°, can be represented by the area between its distribution curve and the axis of temperature; these areas, expressed as % of the total area, are:

	Carbinol: CHMePh·OH.	CHEtPh·OH.	CHPr^{n}Ph·OH.
S_Ni	41·3	43·2	62·4
S_N2	23·6	22·1	17·6
S_N1	35·2	34·6	20·0

With increasing electron release by the methyl, ethyl, and *n*-propyl groups, the domain of S_N2 diminishes, that of S_Ni is markedly increased, and that of S_N1 decreases. The last two mechanisms are both known to be promoted by electron release by groups attached to the carbon atom undergoing substitution (Hughes, Ingold, *et al.*, *locc. cit.*), and it is inferred that when, as here, conditions permit these two mechanisms to compete, S_Ni is promoted more strongly by electron release than is S_N1. This can be seen in the region 0° to +20° in Figs. 1 and 2; here S_N2 is of relatively little importance and, with increasing electron release, S_Ni competes successfully with S_N1. This situation is entirely compatible with the known effect of electron release in promoting S_N1 over S_N2 when these two mechanisms compete in the absence of S_Ni.

The author wishes to thank Drs. J. Kenyon, M. P. Balfe, and J. W. Smith for reading the MS. of this paper.

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61. *The Structure of Pyrethrolone and Related Compounds. Part II.*

By T. F. WEST.

The methyl ether obtained from pyrethrolone by means of methyl sulphate has $[\alpha]_D^{20°}$ −97·3° (in alcohol), whereas that derived from pyrethrolone semicarbazone by treatment with methyl-alcoholic sulphuric acid is optically inactive (cf. Staudinger and Ruzicka, *Helv. Chim. Acta*, 1924, **7**, 212). The corresponding semicarbazones have m. p. 183°, $[\alpha]_D^{20°}$ −82° (in pyridine), and m. p. 197°, $[\alpha]_D$ ±0° (in pyridine), and the former on treatment with methyl-alcoholic sulphuric acid is converted into the optically inactive pyrethrolone methyl ether obtainable directly from pyrethrolone semicarbazone. Both compounds are shown by light-absorption measurements to contain similar chromophoric systems and are therefore probably stereoisomeric.

The failure of these and other pyrethrolone derivatives to undergo the Diels–Alder condensation is discussed, and an explanation is suggested by postulating a *cis*-configuration—similar to that present in *cis*-piperylene—for a conjugated side chain.

A fresh formulation for pyrethrolone is proposed which appears to be more in keeping with the chemical evidence than the structures previously advanced.

ALTHOUGH the absorption-spectra data appear to indicate that in the pyrethrolone fragment of the pyrethrin molecules two separate light-absorbing components are present, *viz.*, (*a*) an αβ-unsaturated ketonic group in the *cyclo*pentenolone ring and (*b*) a conjugated system in the pentadienyl side chain (Gillam and West, J., 1942, 671; this vol., p. 49; West, this vol., p. 51; cf. LaForge and Acree, *J. Org. Chem.*, 1942, **7**, 418), yet it has been pointed out by LaForge and his co-workers that the chemical evidence is inconclusive. For instance, LaForge and Haller (*ibid.*, 1938, **2**, 546) reported, amongst other divergences from the behaviour expected of a conjugated system, that neither pyrethrolone nor its deoxy-derivative pyrethrone forms addition products with α-naphthaquinone or gives normal adducts with maleic anhydride—the amorphous, resinous substance produced with the latter was considered to be due to the effect of traces of acids in polymerising pyrethrone.

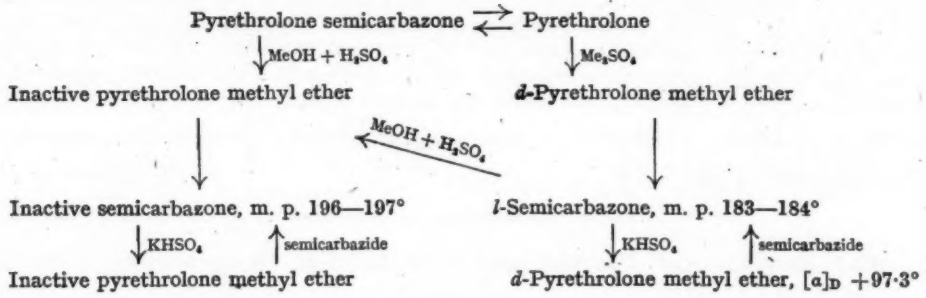
Pyrethrolone methyl ether is comparatively stable towards acids and less easily polymerised than pyrethrolone, and it has therefore been subjected to the action of maleic anhydride and other dienophiles. In the earlier experiments (commenced some years ago) the pyrethrolone methyl ether was prepared from pyrethrolone by treatment with methyl sulphate, as recommended by Staudinger and Ruzicka (*loc. cit.*, p. 224), but in confirmatory work it was decided to use, in addition, for comparison, samples of the methyl ether prepared by refluxing pyrethrolone semicarbazone with sulphuric acid and methyl alcohol (*idem, ibid.*, p. 222). These authors reported that each of these methods gave methyl ethers of which the semicarbazones (m. p. 183°) appeared to be identical. Gnadinger and Corl (*J. Amer. Chem. Soc.*, 1933, **55**, 1221) found that the sample of pyrethrolone methyl ether which they obtained from pyrethrolone semicarbazone yielded a semicarbazone, m. p. 187°, but stated that this high m. p. was "probably because of the presence of a small amount of pyrethrolone."

The author was unable to raise above 184° the m. p. of the semicarbazone obtained from pyrethrolone methyl ether prepared from pyrethrolone and methyl sulphate, whereas samples of the semicarbazone from the ether obtained by treating pyrethrolone semicarbazone with methyl-alcoholic sulphuric acid melted as high as 197°. The two methyl ethers and their semicarbazones exhibited similar absorption spectra and therefore, presumably, contained similar chromophoric systems, but samples of the ether prepared with methyl sulphate had α_D +85°, whereas the second compound was optically inactive. The semicarbazone (m. p. 183°) prepared from the dextrorotatory pyrethrolone methyl ether had $[\alpha]_D$ −82° (in pyridine), whereas that (m. p.

197°) derived from the optically inactive ether was itself inactive. During purification it was noticed that the active was more soluble than the inactive semicarbazone in organic solvents. Neither of the ethers could be reduced by the Pondorff–Meerwein method.

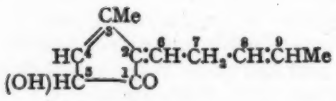
The active ether was recovered substantially unchanged after treatment with methyl-alcoholic sulphuric acid under the conditions used for the preparation of the inactive methyl ether from pyrethrolone semicarbazone. On the other hand, treatment of the lævorotatory pyrethrolone methyl ether semicarbazome in this manner gave an inactive pyrethrolone methyl ether of which the semicarbazone was shown to be identical with that prepared from the inactive methyl ether derived directly from pyrethrolone semicarbazone. Both the dextrorotatory and the inactive pyrethrolone methyl ether were regenerated satisfactorily, from the appropriate semicarbazones by treatment with aqueous potassium hydrogen sulphate in the presence of ether, but the inactive semicarbazone was hydrolysed much less rapidly. The compound regenerated from the semicarbazone of m. p. 183° had $[\alpha]_D$ +97·3° (in alcohol), and this was the highest value observed during the course of the work on these pyrethrolone derivatives.

The relationship between these compounds can be summarised as follows:

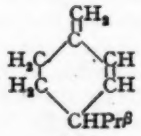


Each of these ethers behaves abnormally in the Diels–Alder reaction. They were recovered unchanged after treatment under various conditions with α-naphthaquinone, *p*-benzoquinone, acetylenedicarboxylic esters, and maleic anhydride, although with the last considerable quantities of polymeric resinous substances were produced. The amount of resinous material was reduced when the reaction was carried out in the presence of phenyl-β-naphthylamine (Craig, *J. Amer. Chem. Soc.*, 1943, **65**, 1011), but although a larger proportion of unchanged methyl ether was recoverable no crystallisable adduct was obtained.

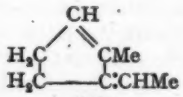
From the ozonolysis evidence, CHR:CH·CH:CHMe appears to be a probable formulation for pyrethrolone (Gillam and West; West; *locc. cit.*), and if the *cis*-configuration is postulated for the pentadienyl side chain, the failure to react in the Diels–Alder condensation can be readily explained (Bachman and Goebel, *J. Amer. Chem. Soc.*, 1942, **64**, 787; Henne and Turk, *ibid.*, p. 826; Craig, *ibid.*, 1943, **65**, 1006). On the other hand, if the side chain $-CH_2{\cdot}CH{:}CH{\cdot}CH{:}CH_2$ for pyrethrolone and its derivatives is considered [the light-absorption data for the conjugated diene obtained by the methods described by Gillam and West (*loc. cit.*) do not allow of a clear differentiation between this structure and –CH:CH·CH:CHMe], this argument does not apply with the same force, although, from a consideration of models, the presence of the terminal substituted methyl group ($\cdot CH_2R$) does seem to permit an explanation involving analogy with the behaviour of *cis*-piperylene. However, an alternative formulation (I) for pyrethrolone has arisen out of discussion of this anomalous behaviour towards dienophiles with Professor A. R. Todd, F.R.S.



(I.)



(II.)



(III.)

The failure of normal reactivity in the Diels–Alder condensation is then readily explained, since the conjugated diene system is locked in the *trans*-position and is analogous with that present in β-phellandrene (II), which gives only polymeric products with maleic anhydride (Goodway and West, *J. Soc. Chem. Ind.*, 1938, **57**, 37; J., 1938, 2028), and in the hydrocarbon of probable formula (III) (King and Robinson, J., 1941, 465) which did not yield an adduct. The formulation (I) explains the production of the main degradation products obtained from pyrethrolone by Staudinger and Ruzicka (*Helv. Chim. Acta*, 1924, **7**, 212) and obviates the interchange of >CO and >CH(OH) groups in the *cyclo*pentenolone ring which is necessary in accommodating the –CH:CH·CH:CHMe side chain. In addition, the behaviour of the pyrethrins on reduction (Haller and LaForge, *J. Org. Chem.*, 1936, **1**, 38; 1937, **2**, 49) and the anomalous behaviour of pyrethrolone derivatives with bromine (LaForge and Haller, *ibid.*, 1938, **2**, 546; Acree and LaForge, *ibid.*, 1940, **5**, 430) appears to find some explanation. If pyrethrone has the same structure as pyrethrolone (I), the fact that the former is optically inactive (LaForge and Haller, *loc. cit.*, 1938) is in agreement with the new formulation. Unfortunately, no light-absorption data are available for compounds containing the system involved in (I).

Experimental.

(Analyses are by Drs. Weiler and Strauss, Oxford.)

Preparation of the d-*Pyrethrolone Methyl Ether.*—Pyrethrolone (6·5 g.) having b. p. 148—150°/0·8 mm., $n_D^{20°}$ 1·5384, $a_D^{25°}$ +8°, $d_{15·5°}^{15·5°}$ 1·051, methyl sulphate (19·5 g.), ether (39 ml.), and potassium hydroxide (19·5 g.) were mixed in this order, and the mixture stirred mechanically for 24 hours. By proceeding essentially as described by Staudinger and Ruzicka (*loc. cit.*, p. 224), the methyl ether obtained (5 g.) had b. p. 114—116°/0·8 mm., $n_D^{20°}$ 1·5130, $a_D^{25°}$ +85° (Found: OMe, 15·9. Calc. for $C_{12}H_{16}O_3$: OMe, 16·1%). A second sample of pyrethrolone (13·4 g.) having b. p. 164—167°/3 mm., $n_D^{20°}$ 1·5370, $d_{15·5°}^{15·5°}$ 1·052, $a_D^{25°}$ 14·1°, $\lambda_{max.}$ 2275 A., ε = 20,200, similarly treated, gave a methyl ether (8·6 g.) having b. p. 122—124°/2 mm., $n_D^{20°}$ 1·5113, $d_{15·5°}^{15·5°}$ 1·004, $a_D^{25°}$ 84·1° (Found: OMe, 15·4%), $\lambda_{max.}$ 2260 A., ε = 23,400. A third sample of the methyl ether prepared by this method had b. p. 104—106°/0·4 mm., $a_D^{20°}$ +85·4°, $d_{15·5°}^{15·5°}$ 1·004 (Found: OMe, 15·7%). Pyrethrolone methyl ether (1·5 g.) (a_D +85°) yielded a semicarbazone (1·83 g.), m. p. 173—176°; recrystallised from methyl alcohol (40 ml.), this (1·4 g.) had m. p. 179°, raised by one further recrystallisation to 183—184° (unchanged by further recrystallisation), $[\alpha]_D$ −79° (*c*, 1·0 in pyridine) (Found: N, 16·7. Calc. for $C_{13}H_{19}O_3N_3$: N, 16·9%); $\lambda_{max.}$ 2320 A., ε = 17,300, and 2665 A., ε = 19,000. The methyl ether (a_D +84·1°) yielded a semicarbazone, m. p. 182—184°, $[\alpha]_D$ −70° (*c*, 0·88 in pyridine), and the third sample (a_D +85·4°) a semicarbazone, m. p. 183—184°, $[\alpha]_D$ −75° (*c*, 0·87 in pyridine). For the preparation of a regenerated *d*-pyrethrolone methyl ether, a mixture of the *l*-semicarbazone (2·8 g.), $[\alpha]_D$ −70° (*c*, 0·76 in pyridine), m. p. 182—184°, potassium hydrogen sulphate (40 g.), water (90 ml.), and ether (90 ml.) in an atmosphere of carbon dioxide was shaken mechanically for 3 days, filtered from unchanged semicarbazone (washed and dried this weighed 1·75 g., m. p. 181—182°), and the ethereal solution washed with brine until neutral. The oil recovered by removing the ether was steam-distilled, and the volatile oil extracted from the distillate with light petroleum. Removal of the solvent gave 0·55 g. of oil which was distilled to give *d*-pyrethrolone methyl ether (0·5 g.), b. p. 87°/0·3 mm., $n_D^{20°}$ 1·5078, $d_{15·5°}^{15·5°}$ 1·001, $[\alpha]_D$ +97·3° (*c*, 16·6 in alcohol) (Found: OMe, 16·7%), $\lambda_{max.}$ 2270 A., ε = 19·300. The semicarbazone prepared from this sample had m. p. 183—184° (after recrystallisation from methyl alcohol), $[\alpha]_D$ −82° (*c*, 0·77 in pyridine).

Preparation of the Inactive Pyrethrolone Methyl Ether (cf. Staudinger and Ruzicka, *loc. cit.*, p. 222).—Pyrethrolone semicarbazone (20 g.), m. p. 208°, was refluxed on the water-bath for 2½ hours with a solution of sulphuric acid (12 g.) in methyl alcohol (200 ml.), then water (400 ml.) was added, and the methyl alcohol removed by distillation under reduced pressure. The solution was cooled, and the solid material filtered off (6·3 g., dry). The filtrate was extracted with light petroleum, and the oil recovered by removing the solvent (7·5 g.) distilled to give a substantially inactive methyl ether (7·2 g.), b. p. 113—115°/0·8 mm., $n_D^{20°}$ 1·5149, $a_D^{25°}$ +0·1° (Found: OMe, 14·3, 14·5. Calc. for $C_{12}H_{16}O_3$: OMe, 16·1%), $\lambda_{max.}$ 2270 A., ε = 24,400. This methyl ether (0·5 g.) yielded a semicarbazone (0·63 g.), m. p. 194°, which recrystallised from methyl alcohol (35 ml.) to give 0·47 g., m. p. 195—196°, unchanged by further recrystallisation; $[\alpha]_D$ ±0° (*c*, 1·0 in pyridine) (Found: N, 16·4. Calc. for $C_{13}H_{19}O_3N_3$: N, 16·9%); $\lambda_{max.}$ 2320 A., ε = 21,000, and 2655 A., ε = 19,000. Mixed with pyrethrolone semicarbazone (m. p. 205°), this had m. p. 182—188° (indef.), and mixed with the *l*-semicarbazone from *d*-pyrethrolone methyl ether (m. p. 183—184°) it had m. p. 182—193° (indef.). After extraction with light petroleum, the filtrate was extracted with ether to give only 0·7 g. of a semicrystalline mass which was added to the 6·3 g. of crystalline material obtained above, and the whole recrystallised from methyl alcohol (300 ml.) to give 5·3 g., m. p. 193—195°. This product was dissolved in methyl alcohol (350 ml.), and the solution filtered and concentrated to 70 ml., whereupon the first crop of crystals (4·5 g.) had m. p. 194—197°; after one recrystallisation from methyl alcohol (210 ml.) the semicarbazone (1·15 g.) had m. p. 195—197°, not depressed by admixture with the semicarbazone, m. p. 195—196°, obtained above; $[\alpha]_D$ ±0° (*c*, 0·74 in pyridine). The filtrate was concentrated by distillation under reduced pressure to 70 ml., whereupon a second crop (2·55 g.) of the same compound, m. p. 195—197°, $[\alpha]_D$ ±0° (*c*, 0·8 in pyridine), was obtained. The filtrate was combined with that from the first recrystallisation above, and the solution concentrated to 30 ml., a further 1·15 g. of crystalline material, m. p. 192° (sintering at 182°), being obtained. This third crop was not further purified.

For the preparation of regenerated *i*-pyrethrolone methyl ether, the inactive semicarbazone (2·85 g.), m. p. 195—196°, was shaken with a mixture of potassium hydrogen sulphate (40 g.), water (90 ml.), and ether (90 ml.) in an atmosphere of carbon dioxide mechanically for 10 days (the inactive semicarbazone was less easily hydrolysed than the *l*-semicarbazone, which was shaken for only 3 days to minimise possible change of the liberated active pyrethrolone methyl ether into the inactive form). The mixture was then filtered from unchanged semicarbazone, which was washed and dried (1·9 g., m. p. 192—193°), and the ethereal solution treated as described above to give 0·5 g. of steam-volatile oil, which was distilled to give 0·45 g. of the inactive pyrethrolone methyl ether, b. p. 85°/0·2 mm., $n_D^{20°}$ 1·5142, $d_{15·5°}^{15·5°}$ 1·015, $[\alpha]_D$ ±0° (*c*, 14·11 in alcohol) (Found: OMe, 15·9%), $\lambda_{max.}$ 2270 A., ε = 25,000. The semicarbazone prepared from this sample had m. p. 196—197° after recrystallisation from methyl alcohol, $[\alpha]_D$ ±0° (*c*, 0·52 in pyridine).

Conversion of the d- *into the* i-*Form.*—The *l*-semicarbazone (1 g.), m. p. 183—184°, was refluxed with methyl-alcoholic sulphuric acid as described above. After removal of the methyl alcohol there was no solid material in suspension, and accordingly the aqueous solution was extracted with light petroleum and the oil (0·3 g.) obtained was distilled to give 0·25 g., b. p. 99—100°/0·3 mm., $n_D^{20°}$ 1·5093, $[\alpha]_D$ ±0° (*c*, 4·2 in alcohol) (Found: OMe, 16·9%), $\lambda_{max.}$ 2250 A., ε = 22,750. The semicarbazone prepared in the usual manner had m. p. 193° when crude, raised by two recrystallisations from methyl alcohol to 196—197°, not depressed by admixture with the semicarbazone (m. p. 195—196°) prepared directly from the methyl ether obtained from pyrethrolone semicarbazone by this method. (After extraction with light petroleum, the aqueous residue was extracted with ether to give only 0·15 g. of a dark, resinous product which was rejected.) When the *d*-pyrethrolone methyl ether itself (0·5 g.) was treated with methyl-alcoholic sulphuric acid, the ether recovered (0·41 g.) had b. p. 105—107°/0·8 mm., $n_D^{20°}$ 1·5075, $[\alpha]_D$ +90° (*c*, 1·2 in alcohol) (Found: OMe, 17·6%), $\lambda_{max.}$ 2270 A., ε = 20,700. This recovered *d*-pyrethrolone methyl ether (0·17 g.), treated in the usual manner, yielded 0·19 g. of a semicarbazone, m. p. 176—179°, which recrystallised from methyl alcohol to give 0·11 g., m. p. 182—183°, not depressed by admixture with the semicarbazone derived from untreated *d*-pyrethrolone methyl ether.

Treatment of d-*Pyrethrolone Methyl Ether with Maleic Anhydride and Other Dienophiles.*—In a typical experiment, pyrethrolone methyl ether (10 g.), $a_D^{25°}$ +85·4°, maleic anhydride (5 g.), and benzene (67 ml.) were mixed, divided into 10 equal portions, heated in sealed tubes in an atmosphere of nitrogen for 6 hours at 100°, and set aside overnight. The benzene was poured from the resin (which, recovered by dissolving in acetone, weighed 4·5 g. when dried in a vacuum desiccator) deposited in the tubes. The benzene solution was washed with hot water—the first wash causing the liberation of an insoluble, sticky, resinous scum (0·3 g. when dry) from the benzene solution—until the aqueous washings were no longer acid to litmus. The benzene was removed by distillation, and the residual oil steam-distilled. The oil extracted from the distillate with light petroleum was distilled, giving 6 g. of unchanged pyrethrolone methyl ether, b. p. 102—103°/0·3 mm., $n_D^{20°}$ 1·5118, a_D +86°, $d_{15·5°}^{15·5°}$ 1·003 (Found: OMe, 15·6%); its semicarbazone had m. p. 183—184° after recrystallisation from methyl alcohol, not depressed by admixture with that obtained from the methyl ether not subjected to the action of maleic anhydride. The non-steam volatile portion was obtained by cooling the water and filtering off the solid material, which was dried in a vacuum desiccator (0·6 g.). This was extracted with methyl alcohol

to leave 0·12 g. of insoluble resin. The methyl alcohol was allowed to evaporate at room temperature, but left only sticky, non-crystalline, resinous material which could not be induced to crystallise by treatment with other solvents. This experiment is typical of a number in which the time of heating was varied, the residue obtained from the benzene solution after washing with water was subjected to vacuum distillation, and the benzene solution was distilled without treatment with water, but in each case only unchanged methyl ether was obtained, yielding the semicarbazone, m. p. 183°, and the resinous higher-boiling material could not be crystallised and decomposed on attempted vacuum distillation. When acetone was employed in place of the benzene as solvent, all the reaction products were retained in the solution, which became viscous, but on distillation, although a smaller proportion of the unchanged methyl ether was recovered, the resinous reaction product could not be distilled or crystallised. When benzene was used as solvent, the deposition of resin in the tubes during heating could be avoided by adding phenyl-β-naphthylamine to the reaction mixture, but in spite of a number of experiments no crystallisable or volatile products (other than unreacted methyl ether) could be obtained. In these experiments the $[\alpha]_D$ of the recovered pyrethrolone methyl ether varied between 85° and 95° (in alcohol), and in each case identification was completed by preparation of the semicarbazone and mixed m. p. determination.

When the ether was heated with methyl acetylenedicarboxylate at 145—150° for 5 hours in an atmosphere of nitrogen, only a small amount of brown resinous material was obtained after removal of unreacted pyrethrolone methyl ether and unchanged ester by distillation. Similar results were obtained with the ethyl ester.

The active pyrethrolone methyl ether was recovered practically quantitatively after being heated with *p*-benzoquinone or α-naphthaquinone in alcoholic solution for periods up to 6 hours in sealed tubes at 100°.

Treatment of Inactive Methyl Ether with Maleic Anhydride.—In a typical experiment the inactive pyrethrolone methyl ether (1·5 g.), maleic anhydride (0·75 g.), phenyl-β-naphthylamine (0·37 g.), and benzene (10 ml.) were heated in sealed tubes in a nitrogen atmosphere at 100° for 6 hours. No resin separated in the tube, but on shaking the benzene solution with water a gummy scum separated. After repeated washing with hot water, the benzene was removed, and the oily residue steam-distilled. The oil (0·9 g.) recovered from the distillate by extraction with light petroleum had b. p. 100—101°/0·5 mm., $n_D^{20°}$ 1·5121, $[\alpha]_D$ + 0·76° (*c*, 5 in alcohol), $d_{15·5°}^{15·5°}$ 1·001, and its identity as unreacted pyrethrolone methyl ether was confirmed by the preparation of the semicarbazone, m. p. 196—197°, not depressed by admixture with semicarbazone prepared from the original ether. The non-steam volatile portion was collected, and distilled at 0·3 mm., but only a further small quantity (0·2 g.) of unreacted methyl ether (yielding the appropriate semicarbazone) was obtained (with some phenyl-β-naphthylamine), leaving a resin which decomposed and darkened on attempted distillation. Similar results were obtained in the absence of the phenyl-β-naphthylamine, except that deposition occurred in the tube during heating of the reactants and the amount of pyrethrolone methyl ether recovered was slightly reduced. The use of acetone instead of benzene did not lead to any crystallisable products.

Determinations of absorption spectra were made in ethyl alcohol solution on a Hilger E_3 quartz spectrograph in conjunction with a Spekker photometer.

I am greatly indebted to Professor A. R. Todd, F.R.S., for stimulating discussions, to Dr. A. E. Gillam for his kindness in making available the absorption spectra data, and to the Directors of Messrs. Stafford Allen and Sons, Ltd., for facilities.

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62. *The Course of Autoxidation Reactions in Polyisoprenes and Allied Compounds. Part VIII. The Photo-oxidation of Methyl Elaidate.*

By DONALD A. SUTTON.

Methyl elaidate readily absorbs oxygen at 35° when irradiated by ultra-violet light to form a hydroperoxide-ester as the primary product. The oxidation follows the same course as in the case of methyl oleate, the product yielding on catalytic hydrogenation and saponification a mixture of hydroxystearic acids, of which one was isolated in apparently homogeneous condition. Peroxidation is accompanied by some degree of secondary reaction, which leads, as might be expected, towards scission of the chain.

A SHORT investigation into the photo-oxidation of methyl elaidate was undertaken in order to ascertain whether the course of reaction was essentially the same as that already reported for methyl oleate (Farmer and Sutton, J., 1943, 119). In view of the results recorded in the latter publication, the following experimental method was adopted. Methyl elaidate was shaken in an atmosphere of oxygen at 35° in the light of a mercury-vapour lamp until about 0·2 mol. of oxygen per mol. of ester had been absorbed. The peroxidic oxygen content of the total product was found to be substantially the same as the whole of the oxygen absorbed (maximum peroxide determination error ±5%; maximum gas measurement error ±5%), which indicated that 90% or more of the oxygen taken up still survived as peroxide groups. The whole of the oxidation mixture was then hydrogenated in the presence of Adams's catalyst, in the expectation that the peroxidised material would yield hydroxystearic ester, and the non-oxidised material stearic ester. By saponification with alcoholic alkali, the total hydrogenation product was converted into a mixture of the corresponding acids, the major portion of which was insoluble in cold aqueous alcohol.

From this major portion an attempt has been made to separate the admixed stearic and hydroxystearic acids (proportion *ca.* 4 : 1), which have very similar solubilities in organic solvents, by conversion of the hydroxy-acid into its acetoxy-derivative, followed by fractional crystallisation. The laborious fractional crystallisation was partly successful, serving to isolate about 1·5 g. of crude acetoxy-acid (from 27 g. of original mixed stearic and hydroxystearic acids), which on saponification yielded a mixture of free hydroxystearic acids. Only one of the acids contained in this mixture has been separated in (apparently) homogeneous condition (m. p. 79°). If the mechanism of oxidation of methyl elaidate is that which has been discussed in connection with the linolenic system (Farmer, Koch, and Sutton, J., 1943, 541), the attack by oxygen is likely to have taken place at any or all of the carbon atoms C_8, C_9, C_{10}, and C_{11}, and indeed, Skellon (*J. Soc. Chem. Ind.*, 1931, **50**, 332)

obtained a substance which was possibly 10-hydroxystearic acid by the oxidation of oleic acid in presence of added peroxidic catalyst. It was not possible to determine the position of the hydroxyl group in the acid of m. p. 79° owing to the lack of reference compounds and the known difficulty of separating mixtures of isomeric monohydroxystearic acids (Tomecko and Adams, *J. Amer. Chem. Soc.*, 1927, **49**, 524).

Accompanying the reduced acids was a small quantity of acid material (soluble in cold aqueous alcohol) of higher oxgen content than monohydroxystearic acid. This was not positively identified, but in view of its properties it must be regarded as an oxygenated scission product (? impure ketononoic acid). There is little doubt that this highly oxygenated material was also present in the crude hydroxystearic ester obtained by catalytic hydrogenation of fairly pure methyl oleate peroxide, and this would explain the fact already reported that the hydroxy-ester contained a higher percentage of oxygen than the theoretical, whereas the aqueous alcohol-insoluble acid derived from the ester had the correct oxygen content (Farmer and Sutton, *loc. cit.*).

Experimental.

Oxidation of Methyl Elaidate.—Methyl elaidate (35·7 g.) (Found: C, 77·1; H, 12·2. Calc. for $C_{19}H_{36}O_2$: C, 77·0; H, 12·2%) was oxidised during 7 hours in exactly the way described for methyl oleate (Farmer and Sutton, *loc. cit.*). The uptake of oxygen was 2·2% by weight, and the percentage of peroxidic oxygen 2·3 (method of Bolland *et al.*, *Trans. Inst. Rubber Ind.*, 1941, **17**, 29).

Hydrogenation of the total product. The product, dissolved in ethanol (75 c.c.), was hydrogenated in the presence of Adams's catalyst. Absorption was rapid, and ultimately 3090 c.c. of hydrogen (760 mm., 17°) were taken up. The theoretical absorption, assuming both the complete utilisation of the oxygen absorbed for hydroperoxide formation and the survival up to the time of hydrogenation of all the original unsaturation, was 3400 c.c.

Saponification of the mixed esters. The ethanol was removed from the hydrogenation product by evaporation in nitrogen on a steam-bath, the residue taken up in benzene (100 c.c.), and the catalyst removed by centrifuging. The benzene was completely removed at reduced pressure, and the residue taken up in rectified spirit (75 c.c.) containing potassium hydroxide (10 g.) and refluxed for 3 hours. The resulting solution was poured into water, cooled, and acidified with 2N-sulphuric acid.

Examination of the mixed acids. The precipitated acid was filtered off, washed with water, and dried (yield 32·8 g.). The aqueous-alcoholic solution was continuously ether-extracted during 3 days, the extract being evaporated to yield 1·0 g. of a low-melting hygroscopic solid (Found: C, 59·6, 59·3; H, 9·5, 9·2; equiv., 171. Calc. for $C_9H_{16}O_2$: C, 62·0; H, 10·4%; equiv., 174·3. Calc. for $C_8H_{14}O_2$: C, 62·7; H, 9·4%; equiv., 172·3). 27 G. of the precipitated acid, which contained much stearic acid (Found: C, 74·9, 74·75; H, 12·5, 12·4; equiv., 283), were boiled with a large excess of acetic anhydride for 4 hours. The bulk of the acetic anhydride was removed by distillation, and the residue of acid anhydrides boiled with water to re-form the corresponding acids. The mixture of the latter was fractionally crystallised three times from acetone, the most soluble portion, chiefly acetoxy-acids, being kept at each operation. In this way 1·5 g. of soluble acids were segregated, but the separation of acetoxy-acids thus achieved was far from complete. The crude mixture of acetoxystearic acids was saponified with alcoholic potash to regenerate the corresponding hydroxy-acids, which were further crystallised from acetone to remove the residue of the stearic acid. The hydroxy-acids (total 1 g.) were again fractionally crystallised from acetone containing a little water to yield a least-soluble portion (colourless prisms, m. p. 79°; 0·3 g.), which consisted of an apparently homogeneous monohydroxystearic acid [Found: C, 71·85; H, 11·95; OH, 11·5; equiv., 300·2. Calc. for $C_{18}H_{36}O_3$ (2 active H atoms); C, 71·9; H, 12·1; OH, 11·3%; equiv., 300·5]. The mother-liquors yielded on further partial evaporation a hydroxy-acid (0·6 g.) of unsharp m. p. 30—50°.

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63. *Molecular Compounds between Amines and Sulphur Dioxide. A Comment on Jander's Theory of Ionic Reactions in Sulphur Dioxide.*

By L. C. Bateman, E. D. Hughes, and C. K. Ingold.

Jander's theory of ionic reactions in liquid sulphur dioxide (cf. *Ann. Reports*, 1939, **36**, 141) is based essentially on (*a*) the occurrence of reactions in which chlorine is displaced as chloride ion from thionyl chloride by means of other anions including sulphite ion, and (*b*) a supposed demonstration that amine–sulphur dioxide addition compounds have the structure $[(R_3N)_2SO]^{++}[SO_3]^{--}$. Our knowledge of the mechanisms available in substitution processes suffices to show that argument (*a*) does not necessitate the theory; and the chemistry on which argument (*b*) is based is incorrect. For instance, Jander has mistaken triethylammonium hydrogen sulphite for the triethylamine–sulphur dioxide addition compound, and his various supposed demonstrations of exchange or oxidation of the sulphite ion, without disturbance to the SO group presumed to be contained in the cation, are all quite illusory. The real addition compound of triethylamine and sulphur dioxide is deeply coloured in the liquid state and in solution. It is a weak electrolyte in sulphur dioxide. Its molecular weight in sulphur dioxide, as determined by elevation of the boiling point of the solvent, corresponds to the simple formula R_3NSO_2. It reacts easily with water and oxygen to form colourless triethylammonium salts, chiefly the hydrogen sulphite, hydrogen sulphate, and normal sulphate. Its constitution is discussed, particularly in relation to Weiss's electron-transfer theory of certain types of molecular compound.

Jander and his collaborators have advanced a comprehensive theory of reactions in liquid sulphur dioxide (Jander and Wickert, *Z. physikal. Chem.*, 1936, *A*, **178**, 57; *Ber.*, 1937, **70**, 251; Jander and Ullmann, *Z. anorg. Chem.*, 1937, **230**, 405; Jander, Knoll, and Immig, *ibid.*, **232**, 229; Jander and Immig, *ibid.*, **233**, 295; Jander and Ruppolt, *Z. physikal. Chem.*, 1937, *A*, **179**, 43). The theory appears to have gained at least a limited degree of

acceptance; Emeléus, for example, characterises it as " convincing " (*Ann. Reports*, 1939, **36**, 141; cf. Emeléus and Anderson, " Modern Aspects of Inorganic Chemistry," p. 482). According to this theory, ions are created in liquid sulphur dioxide by the transference of an oxide ion (O^{--}), much as they are created in water by the transference of a proton. There is the difference that the ions formed by oxide-ion transfer are bivalent, but, this being understood, the ionic equilibrium assumed for sulphur dioxide, $2SO_2 \rightleftharpoons SO^{++} + SO_3^{--}$, is compared with the fundamental ionic equilibrium of aqueous solution: $2H_2O \rightleftharpoons H_3O^+ + OH^-$. It is implied that, for reactions in sulphur dioxide, ionised sulphites are " analogous to bases," *i.e.*, that they play a rôle which is similar, apart from the valency difference, to that of ionised hydroxides in aqueous reactions. Likewise, the assumed cation SO^{++} is supposed to function, except as to its valency, similarly to the hydrogen ion of aqueous solutions.

The arguments by which Jander seeks to establish this concept are of two general kinds, which it is convenient to distinguish since they are to be criticised on different grounds.

Argument (1) has reference to the reactions of thionyl chloride with inorganic anions. The central example is the reaction with sulphites, *e.g.*, $Cs_2SO_3 + SOCl_2 \longrightarrow 2SO_2 + 2CsCl$, which Jander regards as a process " analogous to neutralisation "—essentially a combination of SO^{++} with SO_3^{--} to form undissociated solvent.

Argument (2) consists in the claim that amines take up SO^{++} from sulphur dioxide, just as they take up protons from water, to form ammonium ions—diammonium ions in sulphur dioxide, because of the bivalency of SO^{++}. The most fully discussed example is that of triethylamine. Analytical support is claimed for the conclusion that this base exists in sulphur dioxide essentially in the form of the ion-pair (I); that this form can be isolated by evaporation of the solvent as a colourless crystalline salt; and that it can be made to undergo anion exchange without disturbance to the SO group in the cation. Jander compares the formation of ion-pair (I) to the formation of ammonium hydroxides in aqueous solutions of amines.

$$[(NEt_3)_2SO]^{++}[SO_3]^{--} \quad (I.)$$

The obvious comment on argument (1) is that it does not necessitate the theory. Thionyl chloride reacts generally with weakly acidic hydroxyl compounds or their anions to liberate, ultimately, both its chlorine atoms as chloride ions, as well as a molecule of sulphur dioxide. We should certainly expect thionyl chloride to show the same type of reactivity towards the anion of so weak an oxy-acid as the bisulphite ion, the special nature of which provides an evident reason for the formation of the second molecule of sulphur dioxide. It is difficult to see what else could happen.

In some reactions of thionyl chloride with organic hydroxyl compounds it is possible to distinguish a first stage, in which only one chlorine atom of the two in thionyl chloride has become liberated as a chloride ion; the other product is then an organic chlorosulphinate, R·O·SOCl. We still do not know, however, whether, or in what circumstances, this first stage is a single process, or is itself composite. The chlorosulphinate might be formed by a bimolecular reaction between the molecule $SOCl_2$ and the hydroxyl compound or its anion; alternatively, it might arise from a unimolecular reaction in which, first, the thionyl chloride ionises to $SOCl^+$ and Cl^-, and then, in a subsequent fast stage, the cation $SOCl^+$ decomposes the hydroxyl compound or associates with the anion. We are familiar with this type of problem in connexion with the substitution reactions of alkyl and acyl halides*: no one would dogmatise nowadays as to the mechanism of such a reaction simply on the basis of a knowledge of the products.

Although the reaction of thionyl chloride with sulphite ion has not yet been separated into stages, it is natural to suppose that two stages exist corresponding to the liberation as chloride ion of the two chlorine atoms. This being assumed, the entity with which the sulphite ion interacts in the first stage may, as shown above, be $SOCl_2$ in a bimolecular displacement of one chloride ion; or it may be $SOCl^+$, the displacement being of a unimolecular nature; it cannot be SO^{++} as in Jander's theory. Even if the assumption of stages be rejected, it remains true that the nature of the ultimate product of the sulphite reaction is not determinative with respect to mechanism.†

Jander's argument (2) is much more to the point; indeed, our opinion is that it would prove his thesis if the alleged facts were true. However, we consider them erroneous for reasons to be shown.

We became interested in this subject because, at the time of the appearance of Jander's papers, we were planning a study of the kinetics of the reaction between tertiary amines and alkyl halides in sulphur dioxide solution—a study which we subsequently carried a certain distance (cf. J., 1940, 1011). It was, however, necessary as a preliminary measure to ascertain whether, for instance, in sulphur dioxide solution, there really are *two* molecules of amine in each kinetically separate particle containing amine, as formula (I) would represent in the case of triethylamine. The internal evidence in Jander's papers, as well as the previous literature of sulphur dioxide–amine systems, seemed to make it very probable that Jander's conclusions were in error, and this was confirmed as soon as we began to study sulphur dioxide solutions of triethylamine. Our results bear on the general question of the nature of molecular compounds and are published in view of the interest recently evinced by other workers in this subject.

The compound to which formula (I) is assigned by Jander and Wickert was obtained by dissolving triethyl-

* Unimolecular and bimolecular substitutions of *acid* halides have been studied in these laboratories, but the results are not yet published.

† No discussion is included in the text of Jander's reactions between thionyl chloride and other anions, because for these even the nature of the ultimate substitution products is hypothetical.

amine in sulphur dioxide and evaporating the solvent. They describe it as a colourless, crystalline substance of m. p. 73°. The composition $(NEt_3 + SO_2)_n$ is stated to have been established by analysis, but an independent appraisal of the only analysis which is even partially quoted, an estimation of the SO_2 content, is precluded by the omission of an essential figure, *viz.*, the weight of substance which gave the stated *weight* of sulphur dioxide (the percentage content is not given). No evidence of molecular complexity is presented, the value $n = 2$ being assumed without proof.

Our finding is that no colourless, crystalline solid compound having a melting point above the room temperature is formed by the combination of triethylamine and sulphur dioxide alone, although various saline substances can be obtained from mixtures of triethylamine and sulphur dioxide by permitting the absorption of moisture and oxygen. Such absorption, however, naturally alters the composition as found by analysis.

In the absence of moisture and oxygen, triethylamine and sulphur dioxide combine, with considerable evolution of heat, to form an orange-red liquid. On cooling to −80°, this sets to a colourless crystalline solid, which, at a temperature well below 0°, melts to the same strongly coloured liquid. This substance appears to have a small but appreciable vapour pressure at room temperature, possibly an indication of dissociation. Either triethylamine or sulphur dioxide can be gradually withdrawn at low pressure with the aid of a chemical absorbent; and, if the absorbent is selective for one of the volatile components, the other accumulates in the gas space. The composition of the residual orange-red liquid is, however, definite, and corresponds to the formula $(NEt_3 + SO_2)_n$, as determined by estimation of its triethylamine and sulphur dioxide content (p. 247). Its molecular complexity has been determined in sulphur dioxide, in which the compound forms a bright orange-yellow solution. It was first demonstrated that, although this solution has a finite electrical conductivity, this is of such an order of magnitude as to indicate that only a quite small proportion of the solute exists in the form of dissociated ions (p. 247). Next, the apparent molecular weight of triethylamine (weighed as such) in sulphur dioxide was found to be normal (p. 247) by measurement of the elevation of the boiling point of the solvent. Since there is no extensive electrolytic dissociation to be taken into account, this result shows that only one triethylamine molecule is contained in each kinetically independent particle of the coloured compound which constitutes the solute. Finally, the molecular weight in sulphur dioxide of the isolated coloured compound (weighed as such) was determined by the same method: it also was normal to within the error of measurement (p. 247). This established the essentially monomeric character ($n = 1$) of the coloured compound ($NEt_3 + SO_2$) in sulphur dioxide solution.

When this compound is exposed to atmospheric air it absorbs water rapidly, and oxygen more slowly. The first visible effect is due essentially to the absorption of water, which converts the original orange-red liquid into a mass of colourless crystals, having m. p. 74—75°. This appears to be a single compound. It has the composition $(NEt_3 + SO_2 + H_2O)$ as we have proved by estimating the triethylamine content as usual, and the sulphur dioxide content both by quantitative oxidation with bromine and by precipitation as barium sulphate after oxidation with excess of bromine. The last two estimations distinguish water absorption, which does not destroy the reducing power of the sulphur dioxide, from oxygen absorption, which does (p. 247). On account of the agreement in melting point and general physical character, we suppose this compound to be the substance for which Jander claimed to have obtained analyses agreeing with the composition $(NEt_3 + SO_2)$. Obviously it is *triethylammonium hydrogen sulphite*, $[NEt_3H]^+[HSO_3]^-$.

This salt is hygroscopic, but even if kept in dry air it undergoes further change, the melting point at first dropping to 55° or lower and then rising to above 115°. Using the same method of analysis, we showed that these changes are due to absorption of oxygen, the bisulphite radical becoming progressively replaced by bisulphate (p. 247). Specimens melting above 115° were found to consist mainly of triethylammonium hydrogen sulphate $[NEt_3H]^+[HSO_4]^-$, though they still contained appreciable amounts of the hydrogen sulphite.

Specimens which had become oxidised also contained a proportion of normal triethylammonium salts: the triethylamine content was slightly higher than corresponded to the sulphite plus sulphate. This can be readily explained if we assume the contaminant to be normal triethylammonium sulphate, though the analyses do not distinguish the presence of this from that of the corresponding normal sulphite. The conversion of triethylammonium hydrogen sulphite by oxidation into triethylammonium hydrogen sulphate produces a considerably stronger acid, and we may well suppose that this would remove a certain amount of triethylamine from the still unoxidised hydrogen sulphite to form normal triethylammonium sulphate, in addition to sulphurous acid which would become lost. Such a loss of sulphur dioxide, over and above the loss by oxidation, appears clearly in the analytical figures (p. 247).

Jander and Wickert claim to have demonstrated anion exchange in compound (I), without disturbance to the structure of its cation, by the isolation of a salt of structure (II) after double decomposition with potassium bromide:

$$\text{(I.)}\quad [(NEt_3)_2SO]^{++}[SO_3]^{--} \xrightarrow[KBr]{} [(NEt_3)_2SO]^{++}Br_2^- \quad \text{(II.)}$$

The salt is said to have been obtained as a residue on evaporation of the sulphur dioxide solvent after filtration from precipitated potassium sulphite. It is described as a white solid of m. p. 230°.

This claim is clearly untenable in the light of the evidence given above: neither the real addition compound of trithylamine and sulphur dioxide, nor the triethylammonium hydrogen sulphite which Jander mistook for it, could have yielded such a substance. As a matter of fact the claim is just as clearly untenable on the evidence

which Jander and Wickert themselves provide. Amplifying a general statement to the effect that their salt was pure and its analysis satisfactory, the authors refer to two estimations. One was of the sulphur content: it gave the result that a quantity of salt corresponding to a content of 0·2968 g. of triethylamine contained 0·0128 g. of sulphur, a figure which, the authors say, is to be compared with a theoretically calculated sulphur content of 0·0129 g. The theoretical sulphur content has, however, been incorrectly calculated, and should be 0·0470 g. The second estimation was of the bromine content, but any independent appraisal of this is excluded by the omission, once again, of an essential figure. There is no evidence at all that the solid analysed was not impure triethylamine hydrobromide, containing a minor proportion of either triethylammonium hydrogen sulphite or (more probably) the hydrogen sulphate. The melting point of pure triethylamine hydrobromide is given in the literature as 248—250°, and hence the melting point of the residue which Jander and Wickert claimed to be salt (II) is not inconsistent with the interpretation suggested.

Similarly untenable is Jander and Immig's claim to have oxidised compound (I) by means of iodine in sulphur dioxide with the result that the sulphite anion was converted into sulphate or exchanged for iodide, all without affecting the SO group in the cation:

$$I_2 + 2[(NEt_3)_2SO]^{++}[SO_3]^{--} \longrightarrow [(NEt_3)_2SO]^{++}[SO_4]^{--} + [(NEt_3)_2SO]^{++}I_2^{--} + SO_2$$

How much iodine will be absorbed by a solution of triethylamine in sulphur dioxide simply depends on how much water, and how much oxygen, have previously been absorbed from the atmosphere, in fact, on how much hydrogen sulphite ion has been formed by hydration and not destroyed by oxidation.*

The compounds of amines with sulphur dioxide are the subject of a considerable previous literature, which is in general agreement with the preceding conclusions. Prior to Jander's publications, numerous aliphatic, aromatic, and heterocyclic amines had been shown to form coloured, easily dissociable, sulphur dioxide addition compounds, having the two components in 1 : 1 molecular ratio (Michaelis, *Ber.*, 1891, **24**, 745; *Annalen*, 1892, **274**, 192, 208; André, *Compt. rend.*, 1900, **130**, 1714; Korezynski and Glebocka, *Gazzetta*, 1920, **50**, I, 378; Hill, *J. Amer. Chem. Soc.*, 1931, **53**, 2598; Foote and Fleischer, *ibid.*, 1934, **56**, 870; Hill and Fitzgerald, *ibid.*, 1935, **57**, 250). With the addition compounds of the less volatile amines a general tendency had been noted towards the loss of sulphur dioxide preferentially; and also, on exposure to air, towards a concurrent loss of colour. In a more detailed study of these phenomena in the case of the aniline addition compound, ($NH_2Ph + SO_2$), Hill showed that, on exposure to the atmosphere, this substance loses some of its sulphur dioxide, and simultaneously absorbs moisture, forming a mixture of colourless salts, which he identified as the hydrogen sulphite and normal sulphite of aniline. The more recent investigators have concentrated on measurements of the physical properties and phase-equilibria of amine–sulphur dioxide systems (cf. Foote and Fleischer, Hill and Fitzgerald, *locc. cit.*; also Bright and Jasper, *J. Amer. Chem. Soc.*, 1941, **63**, 2486; Bright and Frobenius, *ibid.*, 1943, **65**, 637; Burg, *ibid.*, p. 1629). Burg concluded from a study of the vapour pressure of the trimethylamine–sulphur dioxide system that the simple formulation $Me_3N \rightarrow SO_2$ for the addition compound is to be preferred to Jander's.

The difficulty we have with the simple, covalent formulation of the addition compounds is that it does not seem easy to account on this basis for their strong colour—for instance, that of the triethylamine–sulphur dioxide compound in the liquid state or in solution in sulphur dioxide. The simple formula $R_3N \rightarrow SO_2$, or its equivalent $R_3\overset{+}{N}-\overset{-}{S}O_2$, depicts a zwitterion: it is an internal ammonium sulphinate, in the same sense as that in which an amine oxide is an internal ammonium oxide. The electronic frequencies both of ammonium ions and of sulphinate ion groups are sufficiently far out in the ultra-violet to make it appear improbable that a zwitterion composed from these two groups should show strong visible colour. The coloured compounds are, moreover, weak, though not very weak, electrolytes in sulphur dioxide. Thus the conductivity of triethylamine–sulphur dioxide in, say, 0·025M-solution at −10·75° is of the order ($10^5\kappa = 3$) of 1% of that of a typical strong electrolyte such as tetraethylammonium chloride at the same concentration and temperature ($10^5\kappa = 216$). The suggestion we would make is that the coloured compounds may exemplify the process to which Weiss in particular has called attention, namely, the formation of molecular compounds by partial or complete electron transfer (Weiss, *Nature*, 1941, **147**, 512; *Trans. Faraday Soc.*, 1941, **37**, 780; J., 1942, 245; J., 1943, 462). It is not, of course, a consequence of this hypothesis that the ions $(NR_3)^+(SO_2)^-$, even when fully formed, must become completely dissociated in any particular solvent, since they may (to put it crudely) attract each other more strongly than the solvent attracts each of them: a necessity for a free-energy increase on dissociation will limit the extent of the process. The ions are, of course, also free radicals, and therefore it should be possible to test this suggestion by means of magnetic measurements or the catalysis of ortho–para-hydrogen conversion. The reason we have not done this is that the work here described was performed only to guide our interpretation of another investigation, and was not intended itself to lead to a publication.

Experimental.

Purification of Materials.—A sample of triethylamine from the British Drug Houses was kept for several days over successive fresh samples of solid potassium hydroxide ("AnalaR"). When the latter no longer became moist or brown,

* It seems obvious that a similar series of criticisms will be found applicable to Jander, Knoll, and Immig's findings, which we have not directly reinvestigated, concerning the compound of unsubstituted ammonia with sulphur dioxide. For this they claim to have established the structure $[(NH_3)_2SO]^{++}SO_3^{--}$ by means of a series of reactions, closely similar to those attributed to the corresponding triethylamine derivative.

the amine was boiled for some hours under reflux with fused barium oxide and fractionated. The part having b. p. 89·3—89·7°/758 mm. was kept for one day over sodium, then boiled under reflux over a fresh sample of sodium, and again fractionated. The centre fraction had b. p. 89·6—89·7°/758 mm. Sulphur dioxide, from a fresh, full syphon, was distilled into the first bulb of an all-glass apparatus, where it was dried by means of phosphoric oxide, and distilled through a column of this reagent.

Triethylamine–Sulphur Dioxide.—A well-dried apparatus filled with nitrogen being used, triethylamine (*ca.* 14 c.c.) and sulphur dioxide (*ca.* 5 c.c.) were brought together in a bulb cooled with solid carbon dioxide and alcohol. The temperature was then allowed to rise, and the apparatus was pumped out for some hours with a rotary oil pump. The pressure was increased to atmospheric by the admission of dry nitrogen in order to facilitate the removal of samples. The *molecular compound* is an orange-red, rather viscous liquid at the ordinary temperature; dilute solutions of it in sulphur dioxide are bright orange-yellow, and they may, of course, be prepared either by adding triethylamine to excess of liquid sulphur dioxide or by first preparing the molecular compound as described above [Found : NEt_3, 60·9; SO_2 (by oxidation), 39·1; SO_2 (as $BaSO_4$), 39·5. NEt_3SO_2 requires NEt_3, 61·2; SO_2, 38·8%].

A number of measurements were made of the conductivity of the compound in sulphur dioxide at −10·75°. For this purpose the sulphur dioxide, purified as described above, was subjected to an additional distillation from a little triethylamine; the distillate, which was quite colourless, was then distilled into the conductivity cell. All distillations were non-ebullient, and were carried out in a good vacuum, and in well-dried apparatus. Triethylamine was added directly to the sulphur dioxide in the cell by using a weight pipette. The temperature was maintained by means of a thermostat fitted with an " Electrolux " refrigerating unit, a small heating bulb, and the usual regulating devices. It was found that the conductivity of the sulphur dioxide solvent was too low to be measured with our apparatus, whereas the solutions had small, but quite appreciable, conductivities, which, however, increased slowly with time. The following experiment is typical : Molarity = 0·0237; conductivity readings :

Time after mixing	3 mins.	1·75 hrs.	19·0 hrs.
$10^5 \kappa$ (with κ in ohm^{-1}cm.$^{-1}$)	2·9	3·4	4·0

In spite of many attempts we have failed to trace the cause of this slow increase of conductivity. However, since the design of our conductivity cell was not such as to exclude rigorously all air and moisture (in order to facilitate cleaning and charging, the cell was " loose " and merely stoppered, not sealed on to a vacuum line), we are not disposed to attach any special significance to the effect, for which a minute amount of hydration and oxidation could be held responsible. Thus we regard the earliest reading as the best approximation to the true conductivity of the molecular compound. It seems to be too closely reproducible to be ascribed to an adventitious impurity, although we cannot be dogmatic with regard to the significance of a conductivity of this small order.

The molecular weight of triethylamine in sulphur dioxide was determined ebullioscopically. A Walker–Lumsden apparatus, adapted on obvious lines for use with solvents boiling below room temperature, was employed for the purpose. With 0·54 g. of triethylamine, the following elevations were recorded :

Vol. of solution (c.c.)	7·25	8·30	9·25	12·35
Elevation of b. p.	0·80°	0·60°	0·50°	0·35°

From the graph of the results we find $M = 111$ (calc. for NEt_3 : $M = 101$).

The same method being used, a determination was made of the molecular weight of triethylamine–sulphur dioxide (weighed as such, with precautions against access of air and moisture) in sulphur dioxide solution. With 1·27 g. of triethylamine–sulphur dioxide, the following elevations were obtained :

Vol. of solution (c.c.)	10·20	11·50	12·75	16·50	18·15
Elevation of b. p.	0·92°	0·77°	0·70°	0·50°	0·45°

From the graph of these data we find $M = 151$ (Calc. for NEt_3SO_2 : $M = 165$). The ebullioscopic method, as used by us, was not very precise : we assess the possible error as about 15%.

Hydration and Oxidation of Triethylamine–Sulphur Dioxide.—A sample of the liquid molecular compound was exposed to ordinary air and stirred until the colour just vanished. The dry solid so formed had m. p. 74—75°, and appeared from its analysis to consist of fairly pure *triethylammonium hydrogen sulphite* [Found : NEt_3, 54·9; SO_2 (by oxidation), 35·3; SO_2 (as $BaSO_4$), 36·5. $NEt_3H_2SO_3$ requires NEt_3, 55·2; SO_2, 35·0]. This material was exposed over phosphoric oxide in the vacuum of a good oil pump for 45 minutes. It then had m. p. 68—70° [Found : SO_2 (by oxidation), 34·3%]. The salt is hygroscopic.

The same substance was kept overnight in air at atmospheric pressure over phosphoric oxide. It then had m. p. 55—60°, and according to its analysis contained a considerable quantity of triethylammonium hydrogen sulphate as well as a small proportion of normal triethylammonium salts [Found : NEt_3, 56·7; SO_2 (by oxidation), 23·2; SO_2 (as $BaSO_4$), 36·0%]. After several weeks' keeping in dry air, these processes had gone further. The material now had m. p. 117—120°, and according to its analysis consisted mainly of triethylammonium hydrogen sulphate with minor proportions of the hydrogen sulphite and of normal triethylammonium salts [Found : NEt_3, 53·9; SO_2 (by oxidation), 6·0; SO_2 (as $BaSO_4$), 31·0. Calc. for $NEt_3H_2SO_4$: NEt_3, 50·8; SO_2 (by oxidation), 0·0; SO_2 (as $BaSO_4$), 32·2%].

Some further analyses are quoted for samples of oxidised salts obtained in other experiments, the conditions of preparation varying slightly from case to case. A sample obtained by exposure of the molecular compound in a desiccator over potassium hydroxide with occasional admission of ordinary moist air had m. p. 55–59° and was unusually rich in normal triethylammonium salts, a circumstance which may be correlated with the observation that an odour of triethylamine is produced when the molecular compound is left in an evacuated desiccator over potassium hydroxide [Found : NEt_3, 57·7; SO_2 (by oxidation), 20·2; SO_2 (as $BaSO_4$), 28·5%]. A crust of solid salts skimmed off from the surface of the liquid molecular compound had m. p. 64—67° [Found : NEt_3, 55·3; SO_2 (as $BaSO_4$), 31·4%]. Material taken directly from excess of the liquid molecular compound by diluting it with ether and acetone, filtering off the substance, and washing it with these solvents, was left for some days in a desiccator. It then had m. p. 115—118° [Found : NEt_3, 54·4, 54·3; SO_2 (as $BaSO_4$), 32·0%].

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64. *Sulphanilamide Derivatives.*

By F. S. Spring and E. P. H. Young.

A number of sulphanilamide derivatives carrying an alkyl group substituent at N^1 have been prepared for test as tuberculocides. Preliminary results show that they are inactive in this respect.

Although there is considerable evidence showing that sulphanilamide drugs have no appreciable effect upon the spread of experimental tuberculosis in animals infected in various ways (Smithburn, *Proc. Soc. Exp. Biol. Med.*, 1938, **38**, 574; Kolmer, Raiziss, and Rule, *ibid.*, 1938, **39**, 581; Steinbach and Dillon, *ibid.*, 1939, **41**, 613; Dietrich, *Amer. Rev. Tuberc.*, 1938, **38**, 388; Flippin, Forrester, and Fitz-Hugh, *ibid.*, 1940, **42**, 821; Heise and Steenken, *ibid.*, p. 801), yet it has been repeatedly claimed that sulphanilamide in relatively large doses will retard the dvelopment of experimental tuberculosis (Rich and Follis, *Bull. Johns Hopkins Hosp.*, 1938, **62**, 77; 1939, **65**, 466; Buttle and Parish, *Brit. Med. J.*, 1938, ii, 776; Greey, Boddington, and Little, *Proc. Soc. Exp. Biol. Med.*, 1939, **40**, 418). It is also claimed that sulphapyridine retards the development of experimental tuberculosis, and similar claims have been made for various sulphanilamide derivatives and sulphones (Ballon, Guernon, and Simon, *Amer. Rev. Tuberc.*, 1942, **45**, 217; Ballon and Guernon, *ibid.*, p. 212; Barach, Molomut, and Soroka, *ibid.*, 1942, **46**, 268; Feldman, Hindshaw, and Moses, *Proc. Staff Meet. Mayo Clinic*, 1940, **15**, 695; 1941, **16**, 187; *Amer. Rev. Tuberc.*, 1942, **45**, 212, etc.).

In the present study, a group of sulphanilamide derivatives containing an alkyl group attached to N^1 * have been prepared in order to test their tuberculocidal properties. It was hoped that the introduction of such an alkyl group would lead to greater penetration by the drug of the waxy structure of the tubercle bacillus (cf. Crossley, Northey, and Hultquist, *J. Amer. Chem. Soc.*, 1939, **61**, 2952; Robinson, J., 1940, 505; Steinbach and Duca, *Proc. Soc. Exp. Biol. Med.*, 1940, **44**, 133; Muschenheim, Forkner, and Duerschner, *ibid.*, 1940, **45**, 556; Bergmann and Haskelberg, *J. Amer. Chem. Soc.*, 1941, **63**, 2243). It was appreciated that the bactericidal properties of the parent sulphanilamide would be reduced by the introduction of an alkyl group, but it was hoped that this effect would be offset by the increase in lipoid solubility.

N^1-*Heptadecylsulphanilamide* and NN′-*disulphanilyltetramethylenediamine* were each prepared by the action of acetylsulphanilyl chloride upon the requisite amine, followed by hydrolysis of the N^4-acetyl group, and also by the condensation of *p*-nitrobenzenesulphonyl chloride with the amine, followed by reduction of the nitro-group. Various derivatives of sulphapyridine of the general formula (I), in which R = *n*-C_3H_7, *n*-C_5H_{11}, *n*-$C_{16}H_{33}$, *n*-$C_{18}H_{37}$, and geranyl, together with N^1-2-(6-*methylpyridyl*)-N^1-*octadecylsulphanilamide* have been prepared. Not one of these sulphanilamides has any action upon the tubercle bacillus *in vitro*; furthermore, there appears to be a decline in their inhibitory action upon hæmolytic streptococci as the size of the alkyl group increases.

p-$NH_2 \cdot C_6H_4 \cdot SO_2 \cdot NR$– (I.)

Experimental.

NN′-*Di*-(p-*nitrobenzenesulphonyl*)*tetramethylenediamine*.—Adipamide (4·5 g.) was added to a solution prepared by addition of bromine (10 g.) to a mixture of sodium hydroxide (27·5 c.c.; 33%) and ice (50 g.). The mixture was heated on the steam-bath for 3 hours. The cold solution was then shaken with *p*-nitrobenzenesulphonyl chloride (13·85 g.) in ether (140 c.c.). The aqueous solution was separated, and acidified with 10% hydrochloric acid; the crude product was collected, washed with water, and purified by solution in hot potassium hydroxide solution (12%), followed by precipitation of the filtered solution with acid. After three crystallisations from aqueous acetone, NN′-*di*-(p-*nitrobenzenesulphonyl*)*tetramethylenediamine* was obtained as golden plates, m. p. 201° (Found: C, 41·9; H, 4·2. $C_{16}H_{18}O_8N_4S_2$ requires C, 41·9; H, 3·9%).

NN′-*Disulphanilyltetramethylenediamine*.—(*a*) A suspension of the nitro-compound (0·5 g.) in a mixture of alcohol (225 c.c.) and hydrochloric acid (*d* 1·15; 25 c.c.) was heated under reflux with tin (1 g.) for 1 hour. After standing overnight, the separated tin complex was collected and decomposed by heating with aqueous sodium carbonate solution (10%). The product was collected and crystallised from 80% alcohol (charcoal), yielding the disulphanilyl compound as needles, m. p. 205°, identical with the product prepared by method (*b*).

(*b*) A cooled solution of putrescine, prepared from adipamide (9 g.) as described above, was shaken with acetylsulphanilyl chloride (30 g.) in ether (300 c.c.). The solid separating at the interface was collected (5 g.) and crystallised from methyl alcohol, from which NN′-*di*(*acetylsulphanilyl*)*tetramethylenediamine* separated as platelets, m. p. 233° (sintering at 218°) (Found: C, 49·8; H, 5·6. $C_{20}H_{26}O_6N_4S_2$ requires C, 49·8; H, 5·4%). Hydrolysis was effected by heating under reflux for 1 hour with alcoholic hydrochloric acid (90 c.c. alcohol; 10 c.c. conc. acid). The hydrochloride was collected (microneedles, m. p. 241°), dissolved in aqueous acetone, and the solution neutralised by addition of sodium carbonate solution. Crystallisation of the product from 80% alcohol gave the *disulphanilyl* compound as needles, m. p. 205°, either alone or when mixed with the specimen prepared by method (*a*) (Found: C, 48·2; H, 5·5. $C_{16}H_{22}O_4N_4S_2$ requires C, 48·2; H, 5·5%).

N^1-n-*Heptadecylsulphanilamide*.—(*a*) Heptadecylamine (5 g.) in ether (100 c.c.) was shaken with *p*-nitrobenzenesulphonyl chloride (4·75 g.) in ether (100 c.c.). After removal of the ether, the residue was crystallised from aqueous acetone, from which *heptadecyl*-p-*nitrobenzenesulphonamide* (3 g.) separated as cream-coloured plates, m. p. 90·5° (Found: C, 62·7; H, 9·2. $C_{23}H_{40}O_4N_2S$ requires C, 62·7; H, 9·1%). Reduction of this compound (0·5 g.) with tin and hydrochloric acid as described above gave N^1-*n*-heptadecylsulphanilamide (0·25 g.) as small needles, m. p. 118° either alone or when mixed with the specimen prepared by method (*b*).

(*b*) Condensation of heptadecylamine (25 g.) and acetylsulphanilyl chloride (23 g.) in ether gave N^4-*acetyl*-N^1-n-*heptadecylsulphanilamide* (22 g.), which separated as fine needles from alcohol, m. p. 128° (Found: C, 66·4; H, 9·8. $C_{25}H_{44}O_3N_2S$ requires C, 66·4; H, 9·7%). Hydrolysis of this acetyl derivative with alcoholic hydrochloric acid, followed by treatment with sodium carbonate, gave N^1-n-*heptadecylsulphanilamide* as needles, m. p. 118° (Found: C, 66·9; H, 9·9. $C_{23}H_{42}O_2N_2S$ requires C, 67·3; H, 10·2%).

* The nomenclature is that of Crossley, Northey, and Hultquist, *J. Amer. Chem. Soc.*, 1938, **60**, 2217.

N¹-2-*Pyridyl*-N¹-n-*propylsulphanilamide*.—A solution of freshly distilled 2-*n*-propylaminopyridine (Slotta and Franke, *Ber.*, 1930, **63**, 690) (10 g.) in dry pyridine (50 c.c.) was treated with acetylsulphanilyl chloride (17 g.), added during 30 minutes with stirring. The mixture was heated on the steam-bath for 3 hours and poured in water. The oil was collected and heated under reflux for 3 hours with aqueous-alcoholic sodium hydroxide solution (10%; 100 c.c.). The product was isolated by precipitation with water and crystallised from aqueous methylated spirit, N¹-2-*pyridyl*-N¹-n-*propylsulphanilamide* separating in needles, m. p. 108° (Found: C, 57·6; H, 6·2. $C_{14}H_{17}O_2N_3S$ requires C, 57·7; H, 5·8%).

N¹-2-*Pyridyl*-N¹-n-*amylsulphanilamide*.—2-Aminopyridine (20 g.) in warm dry pyridine (20 c.c.) was treated with sodamide (8·2 g.). After the initial reaction had subsided, *n*-amyl bromide (37 g.) was added slowly, and the mixture set aside for 2 days, then heated under reflux for 3 hours. The cold mixture was poured into aqueous sodium carbonate solution, and the product isolated by means of ether. 2-n-*Amylaminopyridine* (22·2 g.), b. p. 130—135°/12 mm., solidified to a mass of colourless plates, m. p. 43° (Found: C, 73·5; H, 9·7; N, 16·85. $C_{10}H_{16}N_2$ requires C, 73·2; H, 9·8; N, 17·1%). The *picrate* separated in yellow needles from alcohol, m. p. 121° (Found: C, 48·9; H, 4·9. $C_{10}H_{16}N_2,C_6H_3O_7N_3$ requires C, 48·9; H, 4·8%). Freshly distilled 2-*n*-amylaminopyridine (15 g.) in pyridine (50 c.c.) was treated with acetylsulphanilyl chloride (21·5 g.), added during 30 minutes with stirring. The reaction was completed by heating for 2 hours on the steam-bath and pouring into water. N⁴-*Acetyl*-N¹-2-*pyridyl*-N¹-n-*amylsulphanilamide* was obtained as needles, m. p. 83°, from 50% aqueous acetic acid (Found: N, 11·3. $C_{18}H_{23}O_3N_3S$ requires N, 11·6%). Hydrolysis of this acetyl derivative with aqueous-alcoholic sodium hydroxide (7·5%) gave N¹-2-*pyridyl*-N¹-n-*amylsulphanilamide* as needles from alcohol, m. p. 74—75° (Found: N, 13·5. $C_{16}H_{21}O_2N_3S$ requires N, 13·2%).

N¹-2-*Pyridyl*-N¹-*cetylsulphanilamide*.—2-Cetylaminopyridine, prepared by a modification of the method described for 2-*n*-amylaminopyridine, had b. p. 210—220°/12 mm. and solidified to a waxy solid, m. p. 67° (Schering-Kahlbaum, B.P. 265,167, give b. p. 215—225°/2 mm.; m. p. 65—66°). The *picrate* separated in yellow needles from alcohol, m. p. 84° (Found: C, 59·4; H, 7·4; N, 12·9. $C_{21}H_{38}N_2,C_6H_3O_7N_3$ requires C, 59·2; H, 7·5; N, 12·8%). Condensation of 2-cetylaminopyridine (15 g.) with acetylsulphanilyl chloride (11 g.) in the manner described for the *n*-amyl analogue gave N⁴-*acetyl*-N¹-2-*pyridyl*-N¹-*cetylsulphanilamide* (18 g.) as plates from light petroleum (b. p. 80—100°), m. p. 88° (Found: N, 8·6. $C_{29}H_{45}O_3N_3S$ requires N, 8·2%). Hydrolysis of this acetyl derivative with aqueous-alcoholic sodium hydroxide (10%) gave N¹-2-*pyridyl*-N¹-*cetylsulphanilamide* as needles, m. p. 77°, from aqueous alcohol (Found: C, 68·6; H, 9·1; N, 9·2. $C_{27}H_{43}O_2N_3S$ requires C, 68·5; H, 9·1; N, 8·9%).

N¹-2-*Pyridyl*-N¹-n-*octadecylsulphanilamide*.—2-*Octadecylaminopyridine*, prepared from octadecyl chloride and 2-aminopyridine, was obtained as a colourless oil, b. p. 180—185°/0·01 mm., solidifying to a waxy solid, m. p. 66—67° (Found: C, 79·35; H, 12·1; N, 8·5. $C_{23}H_{42}N_2$ requires C, 79·8; H, 12·1; N, 8·1%). Condensation of the base with acetylsulphanilyl chloride, followed by hydrolysis of the acetyl derivative, yielded N¹-2-*pyridyl*-N¹-*octadecylsulphanilamide* as needles from aqueous alcohol, m. p. 70—71° (Found: N, 8·6. $C_{29}H_{47}O_2N_3S$ requires N, 8·4%).

N¹-*Geranyl*-N¹-2-*pyridylsulphanilamide*.—2-Aminopyridine (10 g.), sodamide (4·15 g.), and xylene (50 c.c.) were heated for 1 hour on the steam-bath. The solution was cooled, treated with geranyl chloride (Forster and Cardwell, J., 1913, **103**, 1339) (18·35 g.; b. p. 101°/12 mm.), and the reaction completed by 3 hours' heating at 150°. The cold reaction mixture was treated with water and extracted with xylene. Distillation gave 2-*geranylaminopyridine* as a yellow oil, b. p. 185—190°/12 mm. (Found: C, 78·2; H, 9·5; N, 12·1. $C_{15}H_{22}N_2$ requires C, 78·3; H, 9·6, N, 12·2%). The *picrate* separated from alcohol in fine yellow needles, m. p. 125° (Found: C, 54·8; H, 5·3; N, 15·0. $C_{15}H_{22}N_2,C_6H_3O_7N_3$ requires C, 54·9; H, 5·5; N, 15·25%). Condensation of this base with acetylsulphanilyl chloride in pyridine, followed by hydrolysis of the intermediate acetyl derivative with 10% alcoholic potassium hydroxide, gave N¹-*geranyl*-N¹-2-*pyridylsulphanilamide* as plates from alcohol, m. p. 75—76° (Found: C, 65·8; H, 6·8; N, 11·1. $C_{21}H_{27}O_2N_3S$ requires C, 65·45; H, 7·0; N, 10·9%).

N¹-2-(6-*Methylpyridyl*)-N¹-*octadecylsulphanilamide*.—A mixture of *n*-octadecyl chloride (24 g.) and freshly prepared 6-amino-2-methylpyridine (27 g.) was treated with sodamide (10 g.). The flask and contents became warm and ammonia was evolved. After standing for 2 days, the mixture was decomposed by addition of water, and the product isolated by means of ether. The fraction, b. p. 170—230°/1 mm., was converted into the *picrate*, which separated in yellow platelets, m. p. 101° (15 g.) (Found: C, 60·8; H, 8·1. $C_{24}H_{44}N_2,C_6H_3O_7N_3$ requires C, 61·1; H, 8·0%). Decomposition of the picrate gave the free base, b. p. 205°/0·25 mm., m. p. 46°, which was condensed with acetylsulphanilyl chloride in pyridine solution to give N⁴-*acetyl*-N¹-2-(6-*methylpyridyl*)-N¹-*octadecylsulphanilamide* in plates from light petroleum (b. p. 80—100°), m. p. 84° (Found: C, 68·4; H, 8·7; N, 7·6. $C_{32}H_{51}O_3N_3S$ requires C, 68·9; H, 9·2; N, 7·5%). The acetyl derivative was hydrolysed by heating under reflux with aqueous-alcoholic sodium hydroxide solution (7·5%) for 2 hours. N¹-2-(6-*Methylpyridyl*)-N¹-*octadecylsulphanilamide* separated in waxy plates from aqueous alcohol, m. p. 77—78° (Found: C, 69·6; H, 9·35; N, 8·0. $C_{30}H_{49}O_2N_3S$ requires C, 69·9; H, 9·5; N, 8·2).

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65. *The Isolation of Euphol and α-Euphorbol from Euphorbium.*

By G. T. NEWBOLD and F. S. SPRING.

Two crystalline monohydric alcohols have been isolated by the chromatographic method from euphorbone, an amorphous solid obtained from euphorbium. One of these is identical with α-euphorbol previously isolated in very small yield from the same source by Bauer and Schröder (*Arch. Pharm.*, 1931, **269**, 209). α-Euphorbol, which has been characterised by the preparation of esters, contains at least two double bonds. The second component, *euphol*, probably has the structure $C_{30}H_{50}O$. It is a monohydric alcohol containing two double bonds, one of which is relatively inert. It is tetracyclic and may belong to the triterpene alcohol group.

EXTRACTION of euphorbium, the resinified latex of *Euphorbia spp.*, with light petroleum gives in fair yield a solid product, " euphorbone " (see Bauer and Schenkel, *Arch. Pharm.*, 1928, **266**, 633, where the earlier literature is cited and reviewed). Euphorbone is not homogeneous, and from it, by a tedious process of fractional crystallisation, two alcohols, α-euphorbol, $C_{26}H_{46}O$, m. p. 127—128°, and β-euphorbol, $C_{31}H_{52}O$, m. p. 89—90°, have been isolated (Bauer and Schenkel, *loc. cit.*; Bauer and Schröder, *Arch. Pharm.*, 1931, **269**, 209). The isolation of α-euphorbol from euphorbone (60 mg. from 750 g. of euphorbium) has also been achieved by Schmidt and

Zacherl (*Monatsh.*, 1931, **57**, 177). Although Müller (*J. pr. Chem.*, 1929, **121**, 97) isolated two alcohols from euphorbone, to which the names vitorbol, m. p. 120—125°, and novorbol, m. p. 123·5—124·5°, were given, Bauer and Schröder (*loc. cit.*) conclude that vitorbol is impure α-euphorbol and that novorbol is not homogeneous.

We have isolated two crystalline monohydric alcohols, in good yield and high purity, from euphorbium. Extraction of the drug with acetone gives an amorphous solid which corresponds closely in its properties to euphorbone. Filtration of a solution of this amorphous solid in light petroleum through a column of activated alumina, followed by washing with the same solvent, gives *euphol*. Subsequent washing of the column with benzene gives a second component, which is almost certainly identical with α-euphorbol.

Euphol, m. p. 116°, $[\alpha]_D$ +32°, which forms needles from acetone, is readily esterified to yield *euphyl acetate*, m. p. 109°, $[\alpha]_D$ +41°, and *euphyl benzoate*, m. p. 137—139°. Analyses of the alcohol and its esters give values in good agreement with the formula $C_{30}H_{50}O$ for the parent alcohol; however, it is to be noted that the analytical values do not exclude a number of near homologues (each of which has the general formula $C_nH_{2n-10}O$). Euphol is diethenoid; it gives a yellow coloration with the tetranitromethane reagent and its acetate absorbs two oxygen atoms from an excess of monoperphthalic acid. Of the two ethylenic linkages in euphol, one is unreactive. Euphyl acetate absorbs one mole of bromine to yield *euphyl acetate dibromide*, m. p. 138·5—139·5°, which gives a yellow coloration with the tetranitromethane reagent. Catalytic hydrogenation of euphyl acetate yields *dihydroeuphyl acetate*, m. p. 123·5—124°, which, like euphyl acetate, gives a yellow coloration with tetranitromethane. Dihydroeuphyl acetate absorbs one atom of oxygen from an excess of monoperphthalic acid, and on hydrolysis yields *dihydroeuphol*, m. p. 120°, further characterised as its *benzoate*, m. p. 160—161°. The two double bonds of euphol are not conjugated, since its acetate does not exhibit selective absorption of high intensity above 2200 A.

The homogeneity of euphol has been confirmed by conversion into the acetate, m. p. 109°, and thence into euphyl acetate dibromide, m. p. 138·5—139·5°; debromination of the last compound with zinc dust and acetic acid gives euphyl acetate, m. p. 109°, which is identical in crystalline form, melting point, and solubility with the original acetate. Hydrolysis of euphyl acetate gives euphol, m. p. 116°, again identical with the original specimen.

Although the analytical data obtained for euphol, dihydroeuphol and their esters do not exclude a number of close homologues of $C_{30}H_{50}O$ as possible formulæ for the parent alcohol, analysis of euphyl acetate dibromide appears to limit the possibilities to either $C_{30}H_{50}O$ or $C_{29}H_{48}O$. The amount of euphol which has been available has not been sufficient to allow a determination of the molecular weight by the reliable method of Sandqvist and Bengtsson. Since euphol is diethenoid and has the general formula $C_nH_{2n-9}OH$, it must be tetracyclic. It does not, however, appear to belong to the normal (3-β-hydroxy) sterol group, since it does not give an insoluble digitonide under conditions which lead to the quantitative precipitation of cholesterol. The ease with which euphol is washed from alumina with light petroleum is remarkable, and is in marked contrast to the tenacity with which alumina holds alcohols of the sterol and triterpene groups; this property of euphol probably has a constitutional significance.

The main benzene fraction from the chromatogram readily gave α-euphorbol. It was characterised as its *acetate*, m. p. 124—125°, which, like the parent alcohol, is optically inactive, and by the preparation of *α-euphorbyl benzoate*, m. p. 133—135°. α-Euphorbol gives a yellow coloration with the tetranitromethane reagent, and its acetate absorbs one mole of hydrogen to give dihydro-α-euphorbyl acetate, m. p. 133—135°, which still gives an unsaturation test with tetranitromethane. Hydrolysis of the dihydro-acetate yields dihydro-α-euphorbol, further characterised as its *benzoate*. A comparion of corresponding derivatives of the α-euphorbol prepared by Bauer and co-workers and by us shows that the two preparations are identical:

	Bauer.	This paper.
α-Euphorbol	M. p. 127—128°	M. p. 126—127°
	Optically inactive	$[\alpha]_D \pm 0°$
Dihydro-α-euphorbol	M. p. 133°	M. p. 132·5—134·5°
Dihydro-α-euphorbyl acetate	M. p. 129°	M. p. 133—135°
	—	$[\alpha]_D$ − 15°

The analytical values for carbon recorded by Bauer and Schenkel and by Bauer and Schröder are consistently lower (about 1%) than ours; these authors postulate the formula $C_{28}H_{46}O$ for α-euphorbol. The analytical data obtained on our derivatives are in good consistent agreement with the formula $C_{30}H_{50}O$ for the parent alcohol, but, as in the case of euphol, they do not exclude several near homologues of the general form $C_nH_{2n-10}O$. α-Euphorbyl acetate gives a *dibromide*, m. p. 169—171°, analysis of which appears to be more in agreement with the formula $C_{29}H_{48}O$ for the parent alcohol than with $C_{30}H_{50}O$, but a final decision on this point must be left until larger quantities of α-euphorbol are available.

In agreement with Bauer and Schenkel, we find that α-euphorbol does not give an insoluble digitonide. Furthermore, α-euphorbyl acetate cannot contain a conjugated diene system, since it does not exhibit selective light absorption of any appreciable intensity above 2200 A.

Both α-euphorbol and euphol give deep red colorations with the Liebermann–Burchard reagent, the solutions showing a green fluorescence which is reminiscent of that produced by the tetracyclic triterpene alcohol basseol (Heilbron, Moffet, and Spring, J., 1934, 1583). Experiments are in progress to examine the possible relationship of euphol and α-euphorbol to the triterpene alcohols.

Experimental.

Finely powdered euphorbium resin (500 g.) was extracted with boiling acetone (1 l.) for 2 hours. The orange-coloured liquid was filtered and kept for 48 hours. The white waxy solid (euphorbone) (37 g.), m. p. 105—110°, was collected; it was very soluble in methyl alcohol, acetone, and ethyl acetate, and, although long fine needles separated from a solution in the last solvent, it was not possible to effect a purification by this means, since crystallisation did not set in until the solution was extremely concentrated. Euphorbone separated in long fine needles, m. p. 65—70°, from light petroleum (b. p. 60—80°). The m. p. of this crystalline form was not altered by further crystallisation from the same solvent, and when attempts were made to recrystallise it from acetone, methyl alcohol or alcohol, it separated as an amorphous powder, m. p. between 102° and 110°. When it was heated at 40° in a vacuum, the light petroleum crystallisate lost both solvent of crystallisation and its crystalline form, the m. p. of the heat-treated specimen being 105—107°. This behaviour was observed by Tschirch and Paul (*Arch. Pharm.*, 1905, **243**, 273) and by Schmidt and Zacherl (*loc. cit.*). Euphorbone contains only a trace of saponifiable matter, and a saponified specimen showed the same behaviour towards solvents as the original euphorbone. Attempts were next made to purify euphorbone by acetylation, but again the product separated as an amorphous powder from acetone, alcohol, and ethyl acetate. Although this acetylated product readily absorbed bromine, attempts to isolate a crystalline bromide were equally unsuccessful.

Euphorbone (20 g.) was extracted (Soxhlet) with light petroleum (b. p. 60—80°; 1 l.) for 3 hours. A yellow rubber-like residue (2·5 g.) remained in the thimble. The solution was set aside at room temperature for 24 hours; a resinous deposit (*ca.* 1·0 g.) had then separated; after removal of the clear solution, the resin proved to be insoluble in light petroleum. The solution was filtered through a column of activated alumina (40 × 4 cm.), and the chromatogram washed with light petroleum (b. p. 60—80°), benzene, and ether to give the following fractions:

Fraction.	Solvent.			
I	Light petroleum	1000 c.c.	Trace	
II	,, ,,	500 ,,	0·2 g.;	colourless viscous oil
III	,, ,,	1000 ,,	3·5 g.;	colourless crystalline solid
IV	,, ,,	1000 ,,	3·2 ,,	,, ,, ,,
V	,, ,,	1000 ,,	1·1 ,,	,, ,, ,,
VI	,, ,,	500 ,,	0·2 ,,	,, ,, ,,
VII	Benzene	500 ,,	4·2 ,,	,, ,, ,,
VIII	,,	500 ,,	0·5 ,,	,, ,, ,,
IX	Ether	500 ,,	0·7 ,,	,, ,, ,,

Fraction II could not be obtained crystalline from methyl alcohol, acetone and alcohol as solvents; it was extremely soluble in these solvents. Fraction III separated from acetone in rosettes of well-formed needles, m. p. 114—115°, which after one recrystallisation from the same solvent gave *euphol*, m. p. 116°, not altered by further recrystallisation, $[\alpha]_D^{19.5°}$ +32° ($l = 1$, $c = 1·2$ in chloroform). Fractions IV and V behaved in the same way, giving euphol as needles from acetone, m. p. 116°, undepressed with the specimen from fraction III (Found: C, 84·5, 84·6; H, 12·0, 12·1; active H, 0·27. $C_{30}H_{50}O$ requires C, 84·4; H, 11·8; 1 active H, 0·23%. $C_{29}H_{48}O$ requires C, 84·4; H, 11·7; 1 active H, 0·245%).

Euphyl acetate, obtained by heating the alcohol (1 part) with acetic anhydride (5 parts) and pyridine (5 parts) for 1½ hours on the steam-bath, separated from alcohol in needles, m. p. 109°, $[\alpha]_D^{22°}$ +41° ($l = 1$, $c = 2·1$ in chloroform), $[\alpha]_D^{19°}$ +26° ($l = 0·5$, $c = 6·0$ in pyridine) [Found: C, 82·1; H, 11·0; *M* (Rast), 449. $C_{32}H_{52}O_2$ requires C, 82·0; H, 11·2%; *M*, 469]. The purification of the acetate by crystallisation is much less wasteful of material than the purification of the alcohol. After 18 hours at 35°, the acetate (1 mol.) had absorbed 2·02 atoms of oxygen from an excess of monoperphthalic acid. An attempt to crystallise the product of the reaction was unsuccessful; it still formed a resin after adsorption on activated alumina, followed by elution with chloroform. *Euphyl benzoate*, prepared by heating a solution of euphol (350 mg.) in pyridine (2 c.c.) and benzoyl chloride (2 c.c.) for 2 hours on the steam-bath, crystallised from methanol–acetone in needles, m. p. 137—139°, $[\alpha]_D^{18·5°}$ +59° ($l = 0·5$, $c = 1·0$ in pyridine) (Found: C, 83·5; H, 10·6. $C_{37}H_{54}O_2$ requires C, 83·7; H, 10·3%).

Euphyl Acetate Dibromide.—Euphyl acetate (0·604 g.) was dissolved in ether (3 c.c.) and treated at room temperature with a solution of bromine in acetic acid (3%). The bromine was absorbed instantaneously; when 7·0 c.c. had been added, the solution contained free halogen and absorption ceased (calc. for 1 |═, 6·9 c.c.). The ether was removed under reduced pressure, and the solution set aside overnight. The crystalline product was recrystallised from acetone and then from alcohol, from which the *dibromide* separated in clusters of fine needles, m. p. 138·5—139·5°, $[\alpha]_D^{20°}$ +23·5° ($l = 1$, $c = 1·2$ in chloroform) [Found (for two different preparations): C, 61·0, 60·9; H, 8·3, 8·6; Br, 25·5, 25·7. $C_{32}H_{52}O_2Br_2$ requires C, 61·1; H, 8·3; Br, 25·4%. $C_{31}H_{50}O_2Br_2$ requires C, 60·6; H, 8·2; Br, 26·0%]. The dibromide (350 mg.) was heated under reflux with zinc dust (3 g.), acetic acid (40 c.c.), and water (3 c.c.) for 6 hours. The hot solution was filtered, concentrated to half-bulk, and precipitated with water. The product, isolated by means of ether, was crystallised from acetone, giving euphyl acetate, m. p. 109°, not depressed by an authentic specimen, $[\alpha]_D^{19°}$ +23° ($l = 0·5$, $c = 2·1$ in pyridine).

Dihydroeuphol.—A solution of euphyl acetate (1·2 g.) in ethyl acetate–acetic acid (1 : 1; 150 c.c.) was shaken with hydrogen and platinum (from 350 mg. of PtO_2). The hydrogen absorption was rapid and practically complete after 15 minutes (observed, 60 c.c. at N.T.P.; calc. for $C_{32}H_{52}O_2$: 1 |═, 58 c.c.). After 2 hours the product was isolated and twice crystallised from alcohol, yielding *dihydroeuphyl acetate* in long needles, m. p. 123·5—124°, $[\alpha]_D^{19°}$ +34·5° ($l = 1$, $c = 1·9$ in chloroform) (Found: C, 81·5; H, 11·5. $C_{32}H_{54}O_2$ requires C, 81·6; H, 11·6%). The dihydro-acetate did not absorb bromine and it gave a yellow coloration with tetranitromethane in chloroform; it absorbed 1·08 atoms of oxygen from an excess of monoperphthalic acid (after 18 hours at 35°). Hydrolysis of the dihydro-acetate with 3% alcoholic potassium hydroxide gave *dihydroeuphol*, which formed felted needles from aqueous acetone, m. p. 120°, $[\alpha]_D^{18·5°}$ +34° ($l = 1$, $c = 1·4$ in chloroform) (Found: C, 84·2; H, 12·2. $C_{30}H_{52}O$ requires C, 84·0; H, 12·2%). *Dihydroeuphyl benzoate*, prepared from the alcohol by means of benzoyl chloride and pyridine, separated from acetone–methyl alcohol in blades, m. p. 160—161° (Found: C, 82·7; H, 10·3. $C_{37}H_{56}O_2$ requires C, 83·3; H, 10·6%).

α-Euphorbol.—Fraction VI from the chromatogram was crystallised from acetone. It proved to be a mixture, rosettes of fine needles separating together with more robust prismatic needles. The m. p. was indefinite (90—110°) and purification by crystallisation was not achieved. Fraction VII, however, readily crystallised from acetone to yield a homogeneous mass of hard needles, m. p. 126—127°, not altered by repeated crystallisation from the same solvent. *α-Euphorbol* also separated from methyl alcohol in needles, m. p. 126—127°, $[\alpha]_D^{17°}$ ±0° ($c = 0·7$, $l = 1$, in chloroform) (Found: C, 84·0; H, 11·8. $C_{30}H_{50}O$ requires C, 84·4; H, 11·8%). *α-Euphorbyl acetate*, obtained from the alcohol (1 part) by heating on the steam-bath with acetic anhydride (3 parts) and pyridine (3 parts), separated from acetone in needles, m. p. 124—125°. A mixture of equal parts of α-euphorbyl acetate and euphyl acetate (m. p. 109°) had m. p. 87—94°

and a mixture of equal parts of α-euphorbyl acetate and dihydroeuphyl acetate (m. p. 123—124°) had m. p. 93—98°. α-Euphorbyl acetate gives a deep yellow coloration with tetranitromethane in chloroform; $[\alpha]_D^{16\cdot5°}$ ±0° (l = 1, c = 1·3 in chloroform) (Found: C, 81·8; H, 11·2. $C_{32}H_{52}O_2$ requires C, 82·0; H, 11·2%). *α-Euphorbyl benzoate*, prepared from the alcohol by means of benzoyl chloride and pyridine, separated from acetone–methyl alcohol in needles, m. p. 133—135°, $[\alpha]_D^{18°}$ +15° (l = 0·5, c = 5·3 in pyridine) (Found: C, 83·9; H, 10·5. $C_{37}H_{54}O_2$ requires C, 83·7; H, 10·3%). A mixture of equal parts of α-euphorbyl benzoate and euphyl benzoate (m. p. 137—139°) melted between 105° and 119°.

Dihydro-α-euphorbyl Acetate.—A solution of α-euphorbyl acetate (0·5 g.) in ethyl acetate (25 c.c.) was added to a suspension of platinum (from 200 mg. of PtO_2) in ethyl acetate (25 c.c.). After shaking with hydrogen for 6 hours, the old catalyst was removed, and fresh catalyst (from 100 mg. of PtO_2) added. Shaking in the presence of hydrogen was continued for 15 hours; hydrogen absorption (total, 25·4 c.c. at N.T.P.; calc. for $C_{32}H_{52}O_2$ 1 |, 23·9 c.c.) had then ceased. The product was crystallised from acetone, yielding dihydro-α-euphorbyl acetate as small plates, m. p. 133—135°, $[\alpha]_D^{18°}$ −15° (l = 0·5, c = 6·0 in pyridine); the m. p. was not altered by recrystallisation. Dihydro-α-euphorbyl acetate gave a pale yellow coloration with tetranitromethane in chloroform (Found: C, 81·3; H, 11·5. Calc. for $C_{32}H_{54}O_2$: C, 81·6; H, 11·6%).

Dihydro-α-euphorbol was obtained from the acetate (150 mg.) by refluxing with alcoholic potassium hydroxide solution (20 c.c., 3%) for 2 hours. After three crystallisations from acetone, it formed needles, m. p. 132·5—134·5° (Found: C, 84·1; H, 11·8. Calc. for $C_{30}H_{52}O$: C, 84·0; H, 12·2%).

Dihydro-α-euphorbyl benzoate, prepared from the alcohol by means of benzoyl chloride and pyridine, separated from acetone–methyl alcohol in small prisms, m. p. 132—134° (Found: C, 82·7; H, 10·8. $C_{37}H_{56}O_2$ requires C, 83·4; H, 10·6%).

α-Euphorbyl Acetate Dibromide.—α-Euphorbyl acetate (100 mg.) in ether (2 c.c.) was treated with a solution of bromine in acetic acid (5%). Contrary to the case of euphyl acetate, bromine was only slowly absorbed. A total volume of 0·9 c.c. of bromine solution (1·3 moles) was therefore added, and the solution set aside at room temperature for 1 hour. After removal of ether at room temperature the mixture was freed from acetic acid by standing over potassium hydroxide in an evacuated desiccator. The solid residue was recrystallised five times from acetone, yielding *α-euphorbyl acetate dibromide* as plates, m. p. 169—171° (decomp.) (Found: C, 60·2, 60·2; H, 7·9, 8·3. $C_{32}H_{52}O_2Br_2$ requires C, 61·1; H, 8·3%. $C_{31}H_{50}O_2Br_2$ requires C, 60·6; H, 8·2%). The dibromide gave a light yellow colour with tetranitromethane in chloroform.

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66. *The Properties of Freshly Formed Surfaces. Part II. The Rate of Adsorption of* iso*Amyl Alcohol at the Air–Water Surface.*

By C. C. ADDISON.

The vibrating-jet technique has been applied to the study of the rate at which surface equilibrium of *iso*amyl alcohol–water mixtures is established. Dynamic tensions at surface ages of the order of 10^{-2} second are recorded for various concentrations, and the velocity at which adsorbate molecules migrate to the surface is calculated.

WHEN amphipathic solute molecules are adsorbed from solution to the air–water surface, the surface tension is lowered to a value lying between that for pure water and that corresponding to full adsorption, and is a function of surface age. Although the properties of equilibrium adsorbed layers have been very fully investigated, no case is known in which the rate of development of this equilibrium has been studied. The vibrating-jet technique (see Part I, J., 1943, 535) has now been applied to the study of surfaces to which surface-active molecules are in the process of migrating. The term "surface equilibrium" is used in this work to imply the surface state attained as soon as the concentration of surface-active molecules in the surface is such that migration no longer takes place, and when dipole orientation of adsorbed molecules is complete. This equilibrium is normally achieved within one second, and is distinct from the slow change in surface tension of the type reported by Adam and Shute (*Trans. Faraday Soc.*, 1935, **31**, 204), which takes place with certain long-chain colloidal electrolytes over periods of days after the initial equilibrium has been established. The rate of development of this equilibrium depends (*inter alia*) on the following factors.

1. *Concentration of Surface-active Solute in the Water Phase.*—The excess of concentration at the surface over that in the bulk of liquid can be made up in a concentrated solution by migration of surface-active molecules from the immediate neighbourhood of the surface. The velocity at which this effect operates is determined by the repulsive forces acting between the aqueous medium and the hydrophobic carbon chain, and provided that the required number of surface active molecules be available within a few molecular radii of the surface, equilibrium will be established rapidly. Diffusion from the bulk of the liquid to the region of low concentration immediately below the surface can then take place without influence on surface properties. As concentration decreases, surface equilibrium is progressively more dependent upon migration to the phase boundary of solute molecules from the bulk of the liquid, and the time necessary for the establishment of final equilibrium will increase accordingly.

2. *Length of Carbon Chain.*—The factors controlling dipole orientation are both electrical and mechanical. The mutual attraction between carbon chains at the surface will increase with increasing chain length; the rate of increase of surface pressure (or decrease of surface tension) will therefore be enhanced by increasing chain length although the mechanical resistance to movement, which is also a function of chain length, will operate to counteract this tendency. As to velocity of migration to the surface, the repulsion between the aqueous medium and the hydrophobic carbon chain can be regarded as the restricting influence operating to set up a form of Donnan membrane equilibrium, and thus as the factor determining the distribution of solute

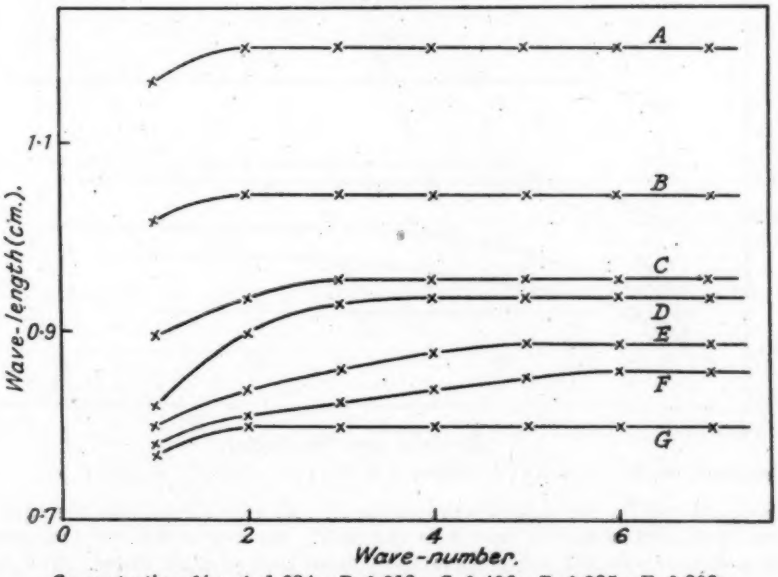
across some arbitrary bounding surface only a few molecular radii from the true physical surface. The speed at which this distribution is completed has been shown to be a function of chain length, and this will be the subject of a later communication.

3. *Nature of Polar Group.*—The electrical attractions and repulsions operating between neighbouring adsorbed molecules have a pronounced effect on equilibrium surface tension. Any body (such as an added ion of opposite charge) capable of affecting these electrical forces modifies the surface tension considerably (Powney and Addison, *Trans. Faraday Soc.*, 1937, **33**, 1253). It is not to be expected, however, that the nature of the polar group will have any appreciable effect on the rate of establishment of dipole orientation; but since the affinity between the aqueous phase and the long-chain carbon compound depends as much upon the nature of the polar group as on the chain length, the effect of the polar group on rate of migration to the surface should be appreciable.

4. *Temperature.*—Although the surface tension of pure liquids falls with increasing temperature, the opposite effect is obtained with solutions of a highly surface-active solute, and this has been explained on the assumption that less solute enters the surface at higher temperatures (*idem*, *ibid.*, p. 1243). Since the liquid is also less viscous, it would be expected that migration into the surface would be accelerated at higher temperatures.

FIG. 1.

iso*Amyl alcohol.*



Concentration, %: *A*, 1·624; *B*, 0·812; *C*, 0·406; *D*, 0·325; *E*, 0·203; *F*, 0·122; *G*, nil.

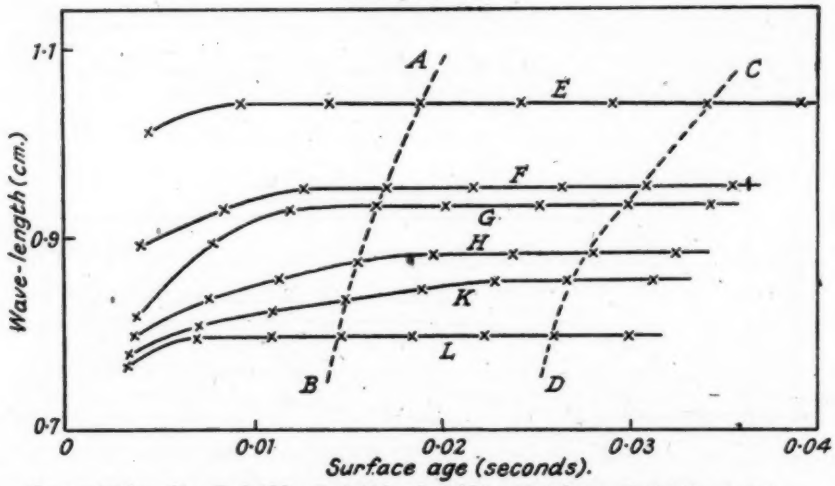
The influence of chain length, concentration, etc., is being studied, but the methods by which the dynamic surface tension, surface age, and migrational velocity are deduced are now recorded, together with their application to only one surface-active substance, *viz.*, *iso*amyl alcohol.

Results and Discussion.—Fig. 1 shows the variation in wave-length which occurs along vibrating jets of water–*iso*amyl alcohol mixtures at 20°. The curves were obtained by using the experimental technique outlined in Part I with a uniform flow rate of 1·990 ml./sec. Since change in surface tension takes place within the first few waves, an improved orifice (described elsewhere) was used which reduced the variations in wave-length at the beginning of a jet of pure liquid from four waves to one wave, and true wave-lengths representative of the surface tension operating at that point in the jet were thus obtained for all but the first wave. Again, as equilibrium is established rapidly, it was not essential to obtain the maximum length of unbroken jet, and the curves shown in Fig. 1 were all obtained by using the jet in a horizontal position. The upper limit for *iso*amyl alcohol concentration is set by its solubility in water at 20°. At the lower concentrations employed, the establishment of surface equilibrium will be largely dependent on migration of *iso*amyl alcohol molecules to the surface from the bulk of the liquid, and for 0·122% alcohol the wave-length changes up to the sixth wave. At a concentration of 0·406% of alcohol, surface equilibrium is reached at the end of the second wave, and at 0·812% the equilibrium is established so rapidly that the wave-length–wave-number curve is similar in form to that for pure water. Adsorption at this and higher concentrations will be complete within the first wave. Since turbulent flow, etc., reduces the length of the first wave below the value corresponding to the operative surface tension, it is not yet possible to measure the tension at a surface age below that corresponding to the end of the first wave, and the 0·812% curve in Fig. 1 represents limiting conditions for the measurement of dynamic tensions.

Since wave-length changes along the jet, wave-number cannot be regarded as proportional to surface age, and it is necessary to determine separately the surface age corresponding to each wave at each concentration. The radius of the circle equivalent in area to the orifice ellipse used was 0·055 cm., giving rise to a linear flow rate of 210 cm./sec. The relation between λ and surface age is given in Fig. 2. For comparison with Fig. 1, points of equal wave-number are connected by the broken lines *AB* and *CD*. In the absence of any adsorption phenomenon, the first wave is smaller than the true value, owing to an overall reduction in average translational velocity rather than to a smaller oscillation period. The latter is probably similar to that given under conditions of true two-dimensional oscillation later in the jet. However, in cases where wave-length is changing due to adsorption, no standard exists on which to assess the extent to which turbulent flow reduces the length of the first wave below its true value. In deducing the results shown in Fig. 2, the age assigned to the jet at the end of the first wave is that determined from the linear velocity under streamlined flow given above, and the measured wave-length. The error involved is of the order of 10^{-4} sec., representing a negligible error of less than 1% on the surface ages under consideration. By using the mathematical treatment given in Part I, the change in surface tension with time has been deduced from the results given in Fig. 2. The length of the first wave cannot be used for calculation of surface tension, and the surface-tension–surface-age relationships

FIG. 2.

*iso**Amyl alcohol.***



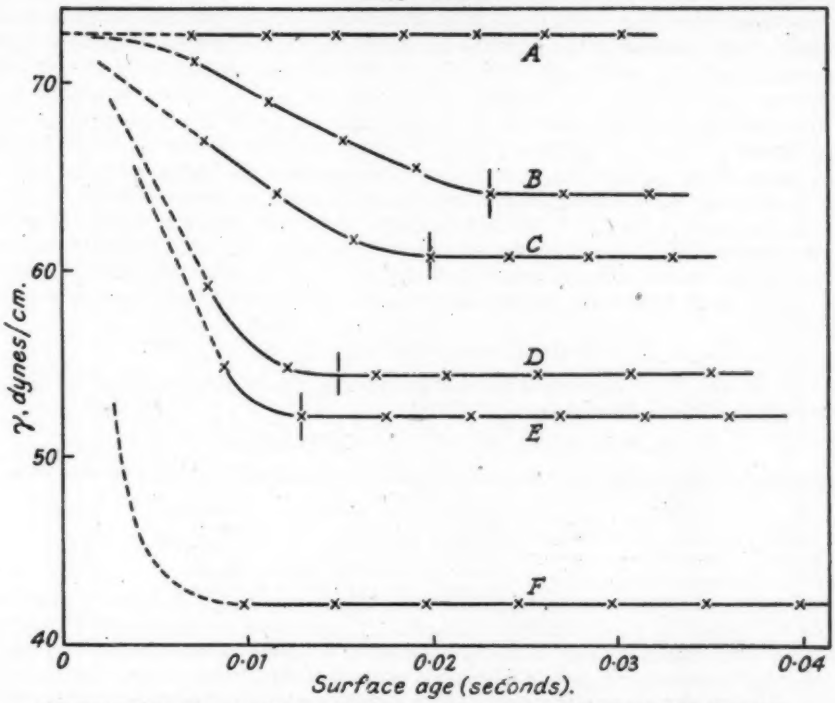
Concentration, %: *E*, 0·812; *F*, 0·406; *G*, 0·325; *H*, 0·203; *K*, 0·122; *L*, *nil*.

shown in Fig. 3 are calculated for the second wave onwards. At zero surface age the surface will contain no higher concentration of alcohol molecules than does the bulk. In view of the low concentrations concerned, the tension at such a surface will not differ appreciably from that of pure water. The curves obtained by calculation (shown as continuous lines in Fig. 3) have therefore been extrapolated (by broken lines) towards $\gamma = 72{\cdot}8$ dynes/cm. for zero surface age. The time required before equilibrium is established is shown in Fig. 3 by short vertical lines cutting the curves; the time required diminishes with increasing concentration.

Sufficient information was not available at this point to decide whether this change of tension with time was due to dipole orientation of the molecules already in the surface or available within a few molecular radii of the surface, or whether the rate of change at different concentrations was a reflection of the rate of migration of molecules far removed from the surface. If the latter case holds true, the surface tensions represented by the level parts of the curves in Fig. 3 should be typical of a surface at which adsorption is complete, and should equal the values obtained by other methods of measurement (such as drop weight, ring method, etc.) in which the surface is at least several seconds old before a measurement can be performed. Fig. 4 shows the relation between surface tension and alcohol concentration at various surface ages. The broken curve *E* shows the probable conditions at 0·005 sec. At all measured surface ages above 0·025 sec. the curve *A* is obtained, and all other curves approach *A* at higher alcohol concentrations. Equilibrium surface tensions for the same system were obtained by the drop-weight technique described by Powney and Addison (*loc. cit.*), Harkins and Brown's correction factors (*J. Amer. Chem. Soc.*, 1919, **41**, 499) being used; 12 seconds were allowed for the formation of each drop. The results obtained are superimposed on curve *A* in Fig. 4. The agreement obtained is almost ideal, and confirms that the surface of the vibrating jet is in a state of final equilibrium as soon as the drift in wave-length ceases, and that migration of surface-active molecules to the surface is then complete. Again, as no breaks have been found in that part of the curves (Fig. 3) covering the period of change, it is probable that with short-chain molecules such as *iso*amyl alcohol the orientation at the surface takes place almost instantaneously. The fall in tension is therefore regarded in this particular case as being dependent on the time necessary to accumulate the required number of active molecules in the surface.

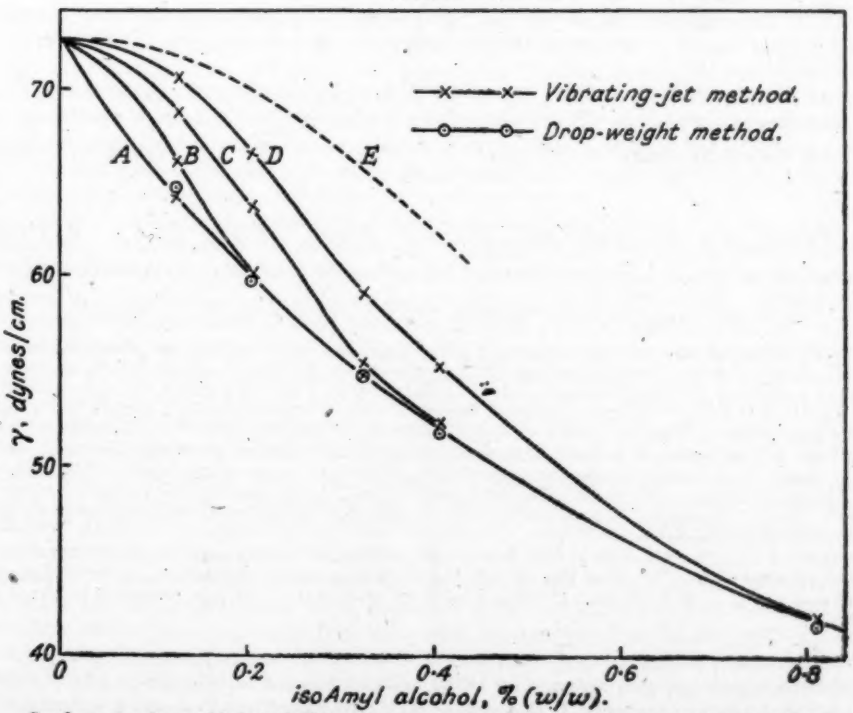
A knowledge of the actual concentration of active molecules at the surface would enable migration to be

FIG. 3.
iso*Amyl alcohol.*



Concentration, %: A, nil; B, 0·122; *C,* 0·203; *D,* 0·325; *E,* 0·406; *F,* 0·812.

FIG. 4.



Surface ages (seconds): A, 0·025 *and over; B,* 0·018; *C,* 0·012; *D,* 0·008; *E,* 0·005.

studied quantitatively. The results set out below are calculated by assuming the applicability of Gibbs's theorem to small molecules, and the migrational velocities given form a valuable basis on which to assess the influence of chain length, etc., on rates of adsorption.

Gibbs ("Scientific Papers," Vol. I, p. 235) deduced the relationship $\Gamma = -\mathrm{d}\gamma/\mathrm{d}\mu$, where Γ is the surface excess of solute, γ the surface tension, and μ the potential of the adsorbed component. In the dilute solutions described here, the activity of the solute may be equated to its concentration c, whence $\Gamma = -c(\mathrm{d}\gamma/\mathrm{d}c)/\boldsymbol{R}T$. The quantity Γ has the dimensions of mass per unit area, and thus represents the mass of adsorbed component actually attached to unit area of the surface; $\boldsymbol{R}$ has the value $83{\cdot}2 \times 10^6$ ($=K$) for 1 g.-mol. of adsorbate, whence $\Gamma = -cM(\mathrm{d}\gamma/\mathrm{d}c)/KT$, where M is the molecular weight of the adsorbate. If the terms of this equation are expressed in c.g.s. units, the value of Γ obtained represents the mass of adsorbate (in g.) attached to 1 cm.^{2} of surface. Since the mass concerned is minute, it can be regarded as being drawn by surface forces from a disc 1 cm.^{2} in cross-section placed with one face in the surface, and having thickness of d cm. Although the distance d is appreciable in terms of molecular dimensions, it is very small in comparison with the diameter of the disc. Surface equilibrium will be complete when the whole of the original solute content of the disc reaches the surface, and the time (t_1 seconds) indicated by experiment as necessary for this equilibrium to be established (Fig. 3) can be equated to the time taken by the last migrating molecules reaching the surface (*i.e.*, those from the opposite face of the disc) to travel d cm. As concentration is expressed in % by weight, then

$$d = 100\,\Gamma/c = -100M(\mathrm{d}\gamma/\mathrm{d}c)/KT$$

The *mean* velocity at which the adsorbate molecules travel to the surface will be termed the "migrational velocity." As this equals d/t_1 cm./sec., then

$$\text{Migrational velocity} = V = -100M(\mathrm{d}\gamma/\mathrm{d}c)/KTt_1$$

Relevant figures used to determine migrational velocities for *iso*amyl alcohol at 20° are given in the table.

c, %	0·122	0·203	0·325	0·406	0·812
$-\mathrm{d}\gamma/\mathrm{d}c$	60	48	36	30	18
$\Gamma \times 10^8$, g./cm.^{2}	2·64	3·52	4·22	4·40	5·28
$d \times 10^6$, cm.	21·7	17·3	13·0	10·8	6·5
t_1, sec.	0·023	0·020	0·015	0·013	<0·01
$V \times 10^4$, cm./sec.	9·4	8·7	8·7	8·4	>6·5

The assumption has been made (see above) that the variation in t_1 with concentration is a result of the varying distances d through which surface-active molecules must migrate to the surface. As a consequence, migrational velocities should be independent of concentration. Any variation of surface tension with time caused by dipole orientation rather than migration would be reflected in discrepant values for migrational velocity. The results in l. 6 of the table indicate that migration is at least the major (if not the only) factor determining surface tension changes over the experimental range of surface ages. The slight fall in velocity which occurs with increasing concentration may be due to mutual interference between migrating molecules, which will also increase with concentration.

The forces producing migration, and the mutual interference, should both increase with increasing chain length. These aspects of the problem will be considered when results over a range of chain length are available.

HARRIS INSTITUTE TECHNICAL COLLEGE, PRESTON. [*Received, February 1st*, 1944.]

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67. *The Triterpene Resinols and Related Acids. Part XVI. A Preliminary Examination of a Major Oxidation Product of the β-Amyrin Group.*

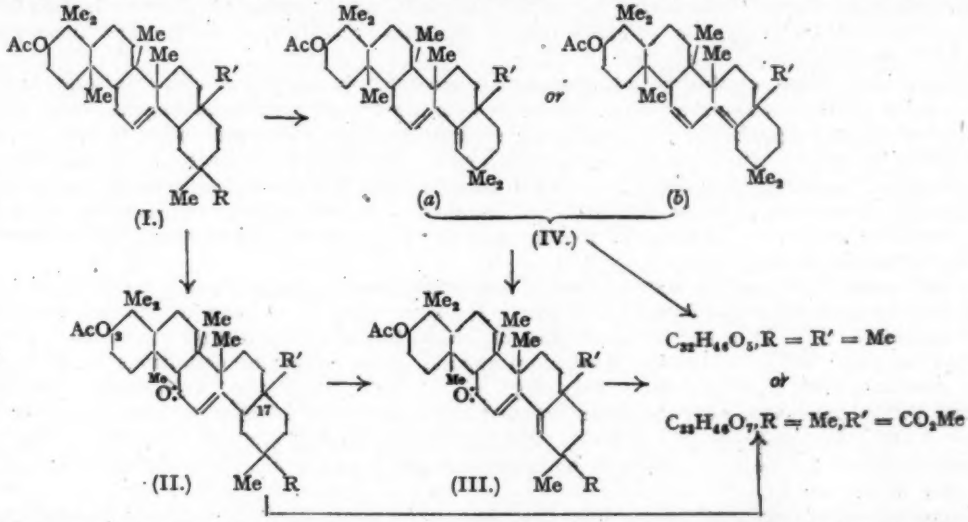
By (the late) NEVILLE MOWER, JAMES GREEN, and F. S. SPRING.

Oxidation of either β-amyrenonyl acetate, $C_{32}H_{48}O_3$ (II, R = R′ = Me), or β-amyradienonyl acetate, $C_{32}H_{46}O_3$ (III, R = R′ = Me), with selenium dioxide gives an acetate, $C_{32}H_{46}O_5$ ("O_5-acetate"), in high yield. Similar oxidation of β-amyrenonyl and β-amyradienonyl benzoates gives the corresponding *benzoate*, $C_{37}H_{48}O_5$. The "O_5-acetate" is also obtained, together with β-amyradienonyl acetate, by a chromic acid oxidation of β-amyradienyl-II acetate (IV*a* or IV*b*, R′ = Me). Treatment of the "O_5-acetate" with potassium hydroxide gives, in high yield, a yellow amorphous product, acetylation of which does not regenerate the parent "O_5-acetate" but yields a crystalline *diacetate*, $C_{32}H_{44}O_5$ (or a near homologue). The "O_5-acetate" is hydrolysed by mineral acid to yield the corresponding alcohol, $C_{30}H_{44}O_4$, reacetylation of which yields the parent "O_5-acetate." The "O_5-acetate" does not contain a hydroxyl group or a reactive carbonyl group and is resistant to catalytic hydrogenation.

This new type of oxidation product is found to be characteristic of the β-amyrin group; treatment either of methyl ketoacetyloleanolate (II; R = Me, R′ = CO_2Me) with selenium dioxide or of methyl acetyldehydro-oleanolate (IV*a* or *b*; R′ = CO_2Me) with chromic acid gives an acetate, $C_{33}H_{46}O_7$, which is an exact analogue of the "O_5-acetate."

THE αβ-unsaturated ketone, β-amyrenonyl benzoate, is obtained by the oxidation of β-amyrin benzoate with chromic acid (Beynon, Sharples, and Spring, J., 1938, 1233; Picard and Spring, J., 1940, 1198). The corresponding acetate, β-amyrenonyl acetate (II, R = R′ = Me), was found to be resistant to oxidation by selenium dioxide (Picard and Spring, J., 1941, 35), using conditions more drastic than those which readily result in the oxidation of β-amyrin acetate (I, R = R′ = Me) to the conjugated β-amyradienyl-II acetate (IV*a* or IV*b*, R′ = Me) (Ruzicka, Müller, and Schellenberg, *Helv. Chim. Acta*, 1939, **22**, 767; Picard and Spring, J., 1941, 35). [The structure (IV*b*) appears to be less satisfactory than (IV*a*) in view of the dehydration of methyl 2-acetyl-

siaresinolate to methyl acetyldehydro-oleanolate (IV; R = CO_2Me) (Bilham, Kon, and Ross, J., 1942, 540); this reaction, however, could involve bond migration. In other respects—behaviour on oxidation (this paper) and catalytic reduction (Ruzicka and Jeger, *Helv. Chim. Acta*, 1941, **24**, 1236)—either formula gives a satisfactory interpretation of the reactions of β-amyradienyl-II acetate.] The introduction of a second ethylenic



linkage into β-amyrenonyl acetate and the corresponding benzoate was readily achieved by Picard and Spring (J., 1941, 35) by a very facile reaction consisting in the treatment of a hot acetic acid solution of the unsaturated ketone with 1 mole of bromine in the presence of a trace of hydrogen bromide. This general reaction has proved to be of great diagnostic value in the triterpene series. When applied to saturated ketones such as β-amyranonyl acetate, methyl ketoacetyldihydro-oleanolate (Picard, Sharples, and Spring, J., 1939, 1045), and α-amyranonyl benzoate (Seymour, Sharples, and Spring, J., 1939, 1075), it gives *iso*-αβ-unsaturated ketones. In the case of " normal " αβ-unsaturated ketones of the series such as β-amyrenonyl acetate (II, R = R' = Me) (or the corresponding benzoate) (Picard and Spring, *loc. cit.*), methyl acetylglycyrrhetate (II; R' = Me, R = CO_2Me) (Ruzicka and Jeger, *Helv. Chim. Acta*, 1942, **25**, 775; Kon and Ross, J., 1942, 741), and ketoacetyloleanolic acid (II; R' = CO_2H, R = Me) (Ruzicka, Jeger, and Winter, *Helv. Chim. Acta*, 1943, **26**, 265), the reaction leads to the formation of the corresponding dienones (III), which exhibit a characteristic absorption spectrum with a maximum at approximately 2800 A. (log ε ≏ 4·1). In view of the relative ease with which bromine partially dehydrogenates β-amyrenonyl acetate, we have reinvestigated the action of various oxidising agents upon this αβ-unsaturated ketone; this paper reports upon the oxidation of β-amyrenonyl acetate and various related compounds with selenium dioxide.

β-Amyrenonyl acetate and β-amyradienonyl acetate are unchanged after prolonged treatment with selenium dioxide in aqueous acetic acid solution. The oxidation of β-amyrenonyl acetate, however, proceeds smoothly if a solution in acetic acid is boiled with selenium dioxide, water being excluded from the reaction mixture. There is thus formed in high yield (*ca.* 70%) an acetate, $C_{32}H_{46}O_5$, m. p. 252—253°. Under these anhydrous conditions, β-amyradienonyl acetate is likewise oxidised in high yield to give the same " O_5-acetate," and β-amyrenonyl benzoate and β-amyradienonyl benzoate give the corresponding *benzoate*, $C_{37}H_{48}O_5$, m. p. 263°.

Ruzicka and Jeger (*Helv. Chim. Acta*, 1941, **24**, 1236) oxidised β-amyradienyl-II acetate (IV*a* or *b*) with chromic acid and obtained an acetate, $C_{32}H_{46}O_5$ (m. p. 256°, corr.), which we find is identical with the selenium dioxide oxidation product from both β-amyrenonyl acetate and β-amyradienonyl acetate. By slight modification of the reaction conditions we also find that chromic acid oxidation of the dienyl-II acetate gives a mixed crystal, m. p. 219—221°, which has been resolved by the chromatographic method into β-amyradienonyl acetate (III, R = R' = Me) and the " O_5-acetate," m. p. 252—253°. The conversion of the dienyl-II acetate into β-amyradienonyl acetate has previously been effected by oxidation with lead tetra-acetate (Ruzicka, Jeger, and Norymberski, *Helv. Chim. Acta*, 1942, **25**, 457).

The " O_5-acetate " is unaffected by prolonged boiling with acetic anhydride and it does not contain any active hydrogen as estimated by the Zerewitinoff method; it cannot contain a free hydroxyl group. It does not contain a reactive carbonyl group, since it is recovered unchanged after heating with alcoholic hydroxylamine acetate at 150° for 3 hours, and it is likewise recovered unchanged after an attempted reduction by the Clemmensen method. In solution in either alcohol or dioxan it does not give a coloration with aqueous ferric chloride and presumably cannot contain a 1 : 2- or 1 : 3-diketone system; furthermore, the " O_5-acetate " does not give the positive Legal reaction characteristic of αβ-unsaturated lactones. In an attempt to determine whether the " O_5-acetate " contains an oxido-group, we found that it is recovered unchanged (*a*) after heating with 2% hydrochloric acid in acetic acid for 3 hours, (*b*) after treatment with 25% hydrogen bromide in acetic acid for 20 hours at 17°, and (*c*) after heating under reflux for 3 hours with 10% hydrogen bromide in acetic acid.

The " O_5-acetate " and the corresponding " O_5-benzoate " are unstable to alcoholic potassium hydroxide, giving a yellow, amorphous, neutral product in high yield, reacetylation of which does not regenerate the original " O_5-acetate " but gives a crystalline *compound*, $C_{33}H_{46}O_5$ (or a near homologue), m. p. 249—251°, in which the presence of two acetyl groups has been demonstrated.

Simple hydrolysis of the " O_5-acetate " is readily accomplished by means of alcoholic hydrogen chloride, which yields the *alcohol*, $C_{30}H_{44}O_4$, m. p. 280·5—281·5°, in almost quantitative yield. The alcohol is readily reacetylated to yield the parent O_5-acetate.

The " O_5-acetate " does not absorb hydrogen, a platinum catalyst being used with ethyl acetate as solvent. However, it gives a faint but definite yellow coloration with the tetranitromethane reagent, which we consider to be of constitutional significance (*i.e.*, indicative of unsaturation), since specimens of the " O_5-acetate " from four different sources all show the same behaviour. The absorption spectrum of the " O_5-acetate " exhibits a band at approximately 2300 A., $\varepsilon = 3000$, together with a low-intensity inflexion at 3000 A. This spectrum we again consider to be of constitutional significance and not due to the presence of an intensely absorbing impurity, since it is exhibited by specimens of the " O_5-acetate " prepared by four different routes and also by the parent alcohol, $C_{30}H_{44}O_4$.

The remote possibility that the angular methyl group attached to C_{17} in β-amyrenonyl acetate (II, R = R′ = Me) may be involved in the formation of the " O_5-acetate " led us to examine the oxidation of methyl ketoacetyloleanolate (II; R = Me, R′ = CO_2Me) in which this angular methyl group is replaced by a carbomethoxyl group. When oxidised with selenium dioxide, under anhydrous conditions, methyl ketoacetyloleanolate gives an *acetate*, $C_{33}H_{46}O_7$, m. p. 253—254°, $[\alpha]_D^{15°}$ +15·9°. Like the " O_5-acetate," this compound gives a faint yellow coloration with the tetranitromethane reagent and exhibits an absorption maximum at 2280 A., $\varepsilon = 3{,}700$. Again like the " O_5-acetate," it is unstable to alkali, but simple hydrolysis of the C_2-acetyl group is accomplished by means of alcoholic hydrogen chloride to give the corresponding *alcohol*, $C_{31}H_{44}O_6$, m. p. 255—256°.

The general nature of the oxidation reactions described in this paper was further demonstrated by treatment of methyl acetyldehydro-oleanolate (IV*a* or IV*b*, R′ = CO_2Me) with chromic acid, the acetate $C_{33}H_{46}O_7$ being obtained. The complete correspondence in behaviour between comparable derivatives of β-amyrin and oleanolic acid shows that the C_{17}-angular methyl group of β-amyrin acetate cannot be involved in the formation of the " O_5-acetate."

From the evidence available it is not possible to formulate a structure for the " O_5-acetate " (and for the corresponding derivative from methyl ketoacetyloleanolate) in terms of the hypothetical structure of β-amyrenonyl acetate (II, R = R′ = Me). In general terms, however, it is to be noted that the oxidation of β-amyradienonyl acetate to the " O_5-acetate " has involved the elimination of two hydrogens and the introduction of two oxygen atoms, this change resulting in a very considerable modification of the original dienone chromophore. This modification does not result in the formation of either an αβ-unsaturated ketone or a 1 : 2-diketone. Of particular significance is the formation of the diacetate, m. p. 249—251°, by hydrolysis of the " O_5-acetate " [which, according to Ruzicka and Jeger (*loc. cit.*), contains one acetyl group *], followed by reacetylation.

In view of its ease of formation in high yield, the elucidation of the structure of the " O_5-acetate " appears to be of importance as a means of testing the hypothetical formula (I) for the β-amyrin group.

Experimental.

Benzoate, $C_{37}H_{48}O_5$.—(*a*) A solution of β-amyrenonyl benzoate (Picard and Spring, J., 1940, 1200) (1·0 g.) in glacial acetic acid (25 c.c.) was heated under reflux with selenium dioxide (1·0 g.). The hot solution was filtered from black selenium, and the latter washed with hot acetic acid; the solvent was removed under reduced pressure, and the solid residue collected and dried. A solution of this solid in benzene–light petroleum (1 : 1, 20 c.c.) was filtered through a column of activated alumina (Brockmann), and the column washed with benzene (20 c.c.). The solvent was removed from the combined filtrates, and the solid crystallised twice from alcohol to give the *benzoate* in colourless needles (0·65 g.), m. p. 262—263°, $[\alpha]_D^{21°}$ +42° ($l = 0·5$, $c = 2·0$). A mixture of equal parts of the new benzoate and β-amyrenonyl benzoate (m. p. 265°) had m. p. 225—229°. The benzoate gives a very pale yellow coloration with tetranitromethane in chloroform (Found: C, 77·8; H, 8·6. $C_{37}H_{48}O_5$ requires C, 77·6; H, 8·45%).

(*b*) Oxidation of β-amyradienonyl benzoate (Picard and Spring, *loc. cit.*) (m. p. 251—252°) was effected as described under (*a*), except that the reaction mixture was heated under reflux for 6 hours. After three crystallisations from alcohol, the product formed fine needles, m. p. 263°, undepressed by the benzoate described under (*a*); $[\alpha]_D^{18°}$ +40° ($l = 0·5$, $c = 2·1$); it gave a faint yellow coloration with the tetranitromethane reagent (Found: C, 77·5, 77·3; H, 8·4, 8·3%).

Acetate, $C_{32}H_{46}O_5$.—(*a*) A solution of β-amyrenonyl acetate (Picard and Spring, *loc. cit.*) (1·0 g.) in glacial acetic acid (30 c.c.) was refluxed with selenium dioxide (1·0 g.) for 20 hours. The solution was filtered hot, fine needles separating from the solution on cooling. After three crystallisations from methyl alcohol the acetate was obtained in needles, m. p. 252—253°, unchanged by further crystallisation; $[\alpha]_D^{23°}$ +35° ($l = 0·5$, $c = 2·1$). By combination and concentration of the mother-liquors a further crop of " O_5-acetate " separated, which on purification attained the constant m. p. 252—253° (total yield, 68%) (Found: C, 75·4; H, 9·3. Calc. for $C_{32}H_{46}O_5$: C, 75·25; H, 9·1%). *Light absorption in alcohol:* Maximum at 2300 A., $\varepsilon = 3600$ with an inflexion at 3000 A., $\varepsilon = 340$.

* (*Note added in proof.*) In order to confirm that the " O_5-acetate " is a monoacetate, it was submitted for a micro-acetyl determination. Surprisingly, the result indicated a diacetate (Found: Ac, 16·7. $C_{32}H_{46}O_5$ requires 1Ac, 8·4; 2Ac, 16·8%.) Employing a macro-method for the estimation of acetyl (using 0·4—0·5 g. of material) we find that the " O_5-acetate " is a mono-acetate (Found: Ac, 8·6, 8·7, 8·6%), and that the micro-estimation of acetyl is untrustworthy in this case. This result raises doubts concerning the " diacetate," m. p. 249—251°, in which the acetyl was estimated by a micro-method; we have not as yet had an opportunity of re-determining the acetyl by a macro-method, but in the meantime it is to be observed that, apart from the acetyl value, the analytical data for this compound are compatible with a *mono*-acetate, $C_{32}H_{46}O_5$, *i.e.*, an isomer of the " O_5-acetate."

(*b*) The oxidation of β-amyradienonyl acetate (Picard and Spring, *loc. cit.*) (100 mg.) with selenium dioxide (80 mg.) was effected as described under (*a*) except that the reaction mixture was refluxed for 8 hours only. After two crystallisations the acetate was obtained in fine needles from methyl alcohol, m. p. 252°, $[\alpha]_D^{23°}$ +35° (*l* = 0·5, *c* = 1·24) (yield, 65%). It was undepressed in m. p. when mixed with the specimen prepared by method (*a*).

(*c*) A solution of chromic anhydride (4·6 g.) in hot stabilised glacial acetic acid (150 c.c.) was added during 30 minutes to a boiling solution of β-amyradienyl-II acetate (Ruzicka, Müller, and Schellenberg, *Helv. Chim. Acta*, 1939, **22**, 767) (5 g.) in glacial acetic acid (360 c.c.), the mixture being stirred and heated under reflux for 1 hour after the addition was complete. The solvent was removed under reduced pressure, and the residue shaken with ether and dilute sulphuric acid. The ethereal solution was washed with sodium hydroxide solution (5%) and with water. Only a trace of acidic matter was obtained by acidification of the alkaline washings. Removal of the solvent from the dried (sodium sulphate) ethereal solution gave a crude neutral fraction (4·0 g.), which crystallised from methanol in needles, m. p. 211—217° (2·2 g.). When recrystallised from the same solvent, the product formed well-defined homogeneous needles, m. p. 219—221°, which remained constant after two further crystallisations (Found : C, 77·0, 77·1; H, 9·2, 9·0. $C_{32}H_{48}O_3$ requires C, 80·0; H, 10·1%. $C_{32}H_{46}O_5$ requires C, 75·25; H, 9·1%). *Light absorption in alcohol :* Maxima at 2290 (ε = 3000) and 2810 A. (ε = 4500). A solution of the neutral fraction (2·0 g.), m. p. 211—217°, in light petroleum (b. p. 60—80°) was filtered through a column of activated alumina (Brockmann ; 30 g.), and the column washed with light petroleum and then with ether, the following fractions being collected :

Fraction.	Solvent, c.c.	Wt. of product, g.	M. p.
1	Light petroleum, 250	0·7	197—204°
2	,, ,, ,,	0·2	216—220
3	,, ,, ,,	0·05	222—244
4	Ether ,,	0·8	247—250
5	,, ,,	nil	—

Crystallisation of fraction 1 from methyl alcohol (from which it separated in needles) was not a satisfactory method of purification, leading to considerable wastage of material and a slow continual increase of the m. p. The top crop and its various mother-liquors were combined, the solvents removed, and the dried residue dissolved in light petroleum (b. p. 40—60° ; 70 c.c.) and filtered through a column of activated alumina (Brockmann ; 30 g.). The column was washed with light petroleum (b. p. 60—80°) and then with light petroleum–benzene (1 : 1 ; 750 c.c.), and the following fractions collected :

Fraction.	Solvent, c.c.	Wt. of product, g.	M. p.
1a	Light petroleum, 250	nil	—
1b	,, ,, 200	0·1	177—181°
1c	,, ,, ,,	0·3	219—226
1d	,, ,, ,,	0·1	225—229
1e	,, ,, ,,	0·05	224—229
1f	Light petroleum–benzene, 250	0·05	220—225
1g	,, ,, ,, ,,	0·05	240—247
1h	,, ,, ,, ,,	nil	—

Fractions 1c, 1d, 1e and 1f were combined (0·5 g.) and crystallised from methanol–acetone to yield fine needles, m. p. 241—250°, which after two recrystallisations from the same solvent gave β-amyradienonyl acetate, m. p. 248—249°, undepressed by an authentic specimen, m. p. 250—251° ; $[\alpha]_D^{15°}$ +338° (*l* = 0·5, *c* = 1·0 in pyridine). *Light absorption in alcohol :* Maximum at 2850 A., ε = 13,000.

Fraction 4 from the original chromatogram was crystallised twice from methanol, from which the acetate, $C_{32}H_{48}O_3$, separated as fine needles, m. p. 252—253°, undepressed by a specimen prepared by method (*a*). It gave a very faint yellow coloration with the tetranitromethane reagent (Found : C, 75·65; H, 8·9. Calc. for $C_{32}H_{46}O_5$: C, 75·25; H, 9·1%). *Light absorption in alcohol :* Maximum at 2285 A., ε = 3300.

(*d*) A solution of chromic anhydride (0·5 g.) in water (0·5 c.c.) was diluted with glacial acetic acid (15 c.c.) and this mixture was added to a boiling solution of β-amyradienyl-II acetate (0·5 g.) in glacial acetic acid (40 c.c.) during 30 minutes. The mixture was refluxed for a further hour and worked up as described under (*c*). The neutral fraction (0·35 g.) was crystallised four times from methyl alcohol to give the " O_3-acetate " in fine needles, m. p. 251—252°, $[\alpha]_D^{22°}$ +32·5° (*l* = 0·5 ; *c* = 1·97). *Light absorption in alcohol :* Maximum at 2300 A., ε = 3800, with a low-intensity inflexion at 3000 A.

" Acid " Hydrolysis of the Acetate, $C_{32}H_{46}O_5$.—The acetate (m. p. 252—253°) (200 mg.) was heated under reflux with methyl-alcoholic hydrogen chloride (5% ; 25 c.c.) for 2 hours ; after the addition of more methyl-alcoholic hydrogen chloride (25 c.c.) the solution was boiled for a further 2½ hours and then concentrated until crystals began to separate and set aside. The crystalline solid was collected (m. p. 274—276°) and recrystallised from methyl alcohol, from which the *alcohol*, $C_{30}H_{44}O_4$, separated in fine needles, m. p. 280·5—281·5°. It was dimorphous, separating as large flat prisms from dilute solutions or as needles when the crystallisation was rapid. Both forms had the same m. p. either alone or when mixed. The alcohol gave a faint yellow colour with the tetranitromethane reagent but no coloration with aqueous ferric chloride (Found : C, 76·9 ; H, 9·2. $C_{30}H_{44}O_4$ requires C, 76·9 ; H, 9·5%). *Light absorption in alcohol :* Maximum at 2280 A., ε = 3000. The alcohol (90 mg.) was set aside at room temperature with acetic anhydride (1 c.c.) and pyridine (2 c.c.) for 14 hours, and then heated on the steam-bath for 15 minutes. The product separated from methyl alcohol as fine needles, m. p. 253°, undepressed by the acetate $C_{32}H_{46}O_5$.

Alkaline Hydrolysis of the Acetate, $C_{32}H_{46}O_5$.—A solution of the acetate (800 mg.) in methyl-alcoholic potassium hydroxide (2 g. of hydroxide, 2 c.c. of water, 18 c.c. of methyl alcohol) was heated in an autoclave at 200° for 15 hours. The yellow solution was diluted with water and extracted with ether, and the ethereal solution washed with water and dried (sodium sulphate). The neutral fraction (550 mg.) obtained after removal of the ether was a yellow oil which could not be obtained crystalline from acetone, methyl alcohol, or alcohol. The dry neutral fraction was treated with acetic anhydride (1 c.c.) and pyridine (1 c.c.) and set aside at 16° for 16 hours. The solution was then heated on the steam-bath for 15 minutes, and the product, which was isolated in the usual manner, crystallised from methyl alcohol–acetone, from which the *diacetate* separated in long fine needles, m. p. 249—251°, not altered by recrystallisation from 95% alcohol, from which the acetate also separated in needles. A mixture of equal parts of the new acetate and the acetate $C_{32}H_{46}O_5$ (m. p. 253°) was completely molten at 223°. The diacetate did not give a coloration with tetranitromethane (in contrast to the acetate $C_{32}H_{46}O_5$) or with aqueous ferric chloride and it did not exhibit selective absorption of any appreciable intensity above 2200 A. ; $[\alpha]_D^{23°}$ +149° (*l* = 0·5, *c* = 0·7) (Found : C, 75·7, 75·7 ; H, 9·0, 9·2 ; Ac, 17·4. $C_{32}H_{46}O_5$ requires C, 75·8 ; H, 8·9 ; 2Ac, 16·4%). The original alkaline solution was acidified with dilute hydrochloric acid, which produced a white opalescence. The mixture was extracted with ether, the ethereal solution washed and dried

(sodium sulphate), and the solvent removed. The residue crystallised from acetone in small prisms. The crystalline solid was collected, washed with ether, and recrystallised from acetone to yield an acid as prisms, m. p. 292—293° (decomp.) after sintering at 290°. The acid did not give a coloration with the tetranitromethane reagent and a solution of the acid in alcohol did not give a coloration with aqueous ferric chloride. An examination of the light absorption properties of an alcoholic solution shows the absence of any selective absorption of appreciable intensity above 2200 A., but this does not preclude the presence of isolated carbonyl groups. The acid was obtained in extremely low yield and its further examination must await attempts to improve this yield.

The neutral diacetate was also obtained in high yield (65%) by hydrolysis of either the " O_5-acetate " or the benzoate, $C_{37}H_{48}O_5$, by heating under reflux with 2% alcoholic potassium hydroxide for 3 hours. In the latter case the neutral fraction was obtained as a yellow amorphous powder which could not be crystallised from aqueous alcohol or acetone. A solution of the neutral fraction (0·6 g.) in benzene–light petroleum (b. p. 60—80°) (1 : 1; 40 c.c.) was filtered through a column of activated alumina (8 × 1 cm.). A yellow zone on the chromatogram gradually moved down the column when it was washed with benzene–light petroleum (1 : 1) (250 c.c.). The colourless filtrate did not contain any material; the column was next washed with benzene (150 c.c.), which eluted the yellow band. Removal of the benzene gave an amorphous yellow solid (0·5 g.) which separated from hot aqueous alcohol as a yellow powder. Acetylation of the powder by heating with acetic anhydride and pyridine for 2 hours on the steam-bath gave the diacetate, in long fine needles from 95% alcohol, m. p. 249—251° (Found: C, 75·7; H, 9·2%). The diacetate, when mixed with the specimen prepared by acetylation of the neutral hydrolysis product of the acetate $C_{33}H_{46}O_5$, showed no depression in m. p.

Methyl Ketoacetyloleanolate.—Ketoacetyloleanolic acid, prepared by the oxidation of acetyloleanolic acid with chromic acid, was obtained from methanol–acetone in fine needles, m. p. 268—270° (yield, 10%). Ruzicka and Cohen (*Helv. Chim. Acta*, 1937, **20**, 804) give m. p. 282—284° (corr.) for this acid, but later Ruzicka, Cohen, Furter, and Sluys-Veer (*ibid.*, 1938, **21**, 1735) recorded m. p. 272—273° (corr.), and Bilham, Kon, and Ross (J., 1942, 539) find m. p. 288° (uncorr.). Methylation of this keto-acid with diazomethane gave methyl ketoacetyloleanolate, which formed prismatic needles from methyl alcohol, m. p. 238—239° (yield, 90%).

The direct oxidation of methyl acetyloleanolate to methyl ketoacetyloleanolate has been described by Kitasato and Sone [*Acta Phytochim.*, 1932, **6**, 213). The oxidation was carried out at 15°, and the product described as prisms, m. p. 224°, later corrected to 237° [*idem, ibid.*, 1933, **7**, 25 (footnote)]. We find that the following modification of the Kitasato and Sone experiment is the best method for the preparation of methyl ketoacetyloleanolate. A boiling solution of methyl acetyloleanolate (5 g.) in glacial acetic acid (45 c.c.) was treated with a solution of chromic anhydride (5 g.) in acetic acid (90%; 50 c.c.), added during 30 minutes with stirring; the solution was then refluxed for 90 minutes. Water (35 c.c.) was added, and the solution set aside overnight. The crystalline (plates) solid was collected and recrystallised from methanol, from which it separated in prisms (1·16 g.), m. p. 238—239°. A solution of this solid in light petroleum (b. p. 60—80°; 50 c.c.) and benzene (40 c.c.) was filtered through a column of activated alumina (Brockmann) (17 cm. × 3 cm.), and the column washed successively with light petroleum–benzene (5 : 4), benzene, and ether, the following fractions being collected:

Fraction.	Solvent, c.c.	Wt. of fraction, g.	M. p.
1	Light petroleum–benzene, 400	nil	—
2	" " " 350	0·17	239—240°
3	Benzene, 300	0·45	240·5—241
4	" "	0·17	240·5—241
5	Ether, 250	0·3	237—238
6	" "	nil	—

Fractions 2, 3 and 4 were combined and crystallised from methyl alcohol–acetone to yield methyl ketoacetyloleanolate (0·62 g.) in prismatic needles, m. p. 240·5—241°, $[\alpha]_D^{17°}$ +66° ($l = 1$, $c = 1·5$ in pyridine). Kitasato and Sone (*loc. cit.*) give m. p. 237° for the ester prepared by the direct oxidation of methyl acetyloleanolate and m. p. 236—238° for a specimen prepared by methylation of ketoacetyloleanolic acid. Ruzicka, Cohen, Furter, and Sluys-Veer, however, record m. p. 252—253° (corr.) for the ester prepared by the latter method. A mixture of the ester, m. p. 240·5—241°, with the ester, m. p. 238—239°, prepared by method (*a*) had m. p. 239—240° (Found for material of m. p. 240·5—241°: C, 75·0; H, 9·8. Calc. for $C_{33}H_{50}O_5$: C, 75·2; H, 9·6%).

Acetate, $C_{33}H_{46}O_7$.—(*a*) Methyl ketoacetyloleanolate (2·3 g.) in glacial acetic acid (30 c.c.) was heated under reflux with selenium dioxide (2·3 g.) for 30 hours. The hot solution was filtered, and the solvent removed from the filtrate, under reduced pressure. The dark red oil crystallised from methanol (charcoal) in needles, m. p. 251—252·5°, which after two recrystallisations from the same solvent gave the *acetate*, $C_{33}H_{46}O_7$, m. p. 253—254°, $[\alpha]_D^{18°}$ +15·9° ($l = 0·5$; $c = 4·6$ in pyridine). The O_7-acetate did not give a coloration with aqueous-alcoholic ferric chloride (Found: C, 71·1, 71·3; H, 8·3, 8·6. $C_{33}H_{46}O_7$ requires C, 71·5; H, 8·5%). *Light absorption in alcohol:* Maximum at 2280 A., $\varepsilon = 3,700$.

(*b*) A boiling solution of methyl acetyldehydro-oleanolate (Ruzicka, Grob, and Sluys-Veer, *Helv. Chim. Acta*, 1939, **22**, 791) (m. p. 224°) (0·9 g.) in glacial acetic acid (35 c.c.) was treated dropwise during 20 minutes with a solution of chromic anhydride (0·9 g.) in acetic acid (90%; 16 c.c.). The solution was refluxed for a further 90 minutes and then evaporated to dryness under reduced pressure. The neutral fraction obtained by extraction with ether was crystallised from methanol to give needles (0·5 g.), m. p. 244—247°. A solution of this solid (0·35 g.) in light petroleum (b. p. 40—60°; 50 c.c.) and benzene (20 c.c.) was filtered through a column of activated alumina (30 g.), and the following fractions collected:

Fraction.	Solvent, c.c.	Weight, g.
1	Light petroleum–benzene (5 : 2), 240	0·015
2	" " " 200	0·010
3	Ether, 200	0·300
4	" "	nil

Fraction 3 was crystallised thrice from methanol to give the acetate, $C_{33}H_{46}O_7$, in fine needles, m. p. 251—252° either alone or when mixed with the specimen described under (*a*). *Light absorption in alcohol:* Maximum at 2280 A., $\varepsilon = 3,600$.

Alcohol, $C_{31}H_{44}O_6$.—The acetate $C_{33}H_{46}O_7$ (160 mg.) was heated under reflux for 4 hours with methanolic hydrogen chloride (5%; 15 c.c.), and the solution concentrated until crystallisation set in. After cooling, the solid was collected and recrystallised from methanol, from which the *alcohol* $C_{31}H_{44}O_6$ separated in small prisms, m. p. 255—256°. The alcohol gave a faint yellow coloration with tetranitromethane in chloroform but no colour with aqueous alcoholic ferric chloride solution; $[\alpha]_D^{16°}$ −3·55° ($l = 0·5$, $c = 2·8$ in pyridine) (Found: C, 72·6; H, 9·0. $C_{31}H_{44}O_6$ requires C, 72·6; H, 8·7%).

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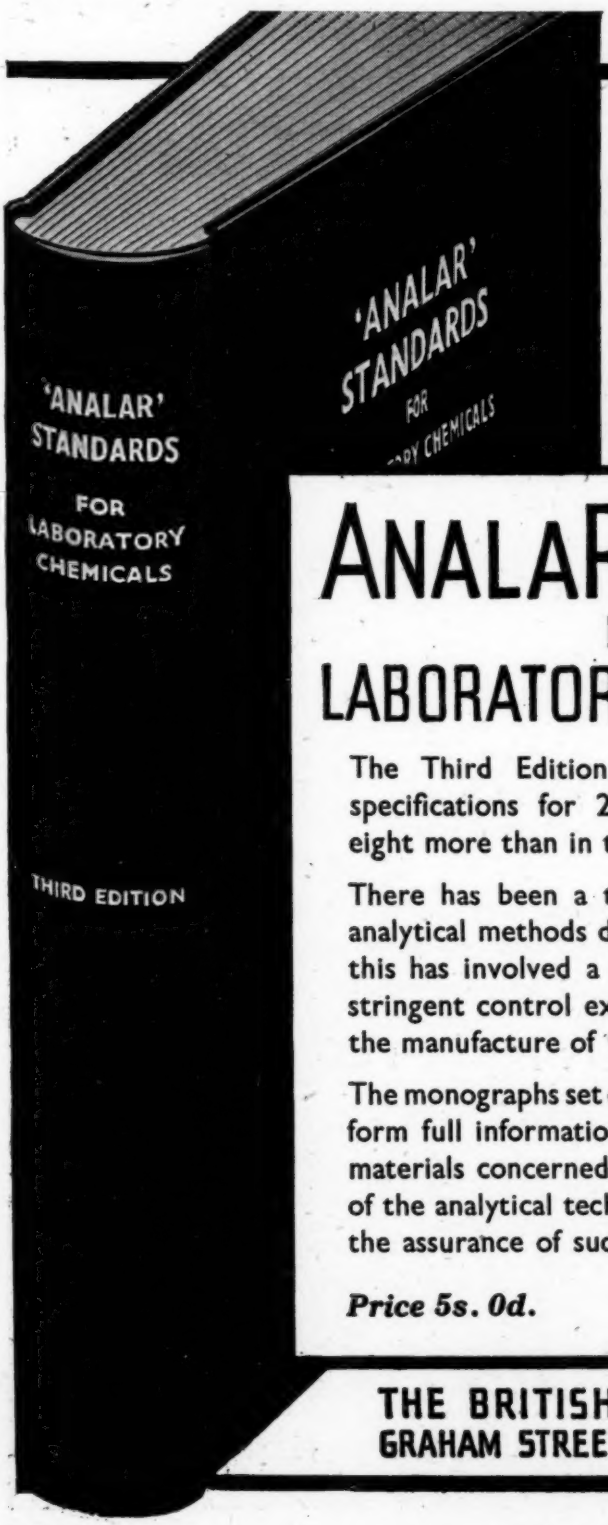

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